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SOAP MICELLES IN NONPOLAR MEDIA

Master´s Programme in Chemical, Biochemical and Materials Engineering
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Abstract

Soaps are amphiphilic molecules (i.e. surfactants), which are combinations of both hydrophobic (fat loving) and hydrophilic (water-loving) components. Surfactants can self-assembly into micelles in aqueous media and reverse micelles (RM) in nonpolar media above a critical micelle concentration (CMC). The understanding of aggregation behavior of soaps and their synergistic effects with other surfactants is crucial for the efficient oil purification. The aim of this thesis was to investigate the CMC of various soaps (i.e. sodium stearate, sodium oleate, calcium stearate and ferric stearate) in rapeseed oil. The effect of free-fatty acids, lecithin and sodium chloride (NaCl) on the CMC of soaps were studied and the effect of soaps on the CMC of lecithin was investigated. The second objective of this thesis was to evaluate how the self-assembly of various micellar systems affect the purification process of oil focusing on filterability. The CMCs of various micellar systems were determined using the TCNQ solubilization method. The CMC of sodium stearate and oleate were 7.1×10^{-5} and 4.4×10^{-5}, respectively. Sodium oleate had slightly lower CMC value, which was concluded to be due to the double bond in its hydrophobic tail. No micelle formation was detected for calcium or ferric stearate in studied concentrations and thus no further experiments were done for these soaps. Even small addition of FFA (5 wt.-%) inhibited the formation of sodium soap micelles, probably because FFA increased the solubility of soaps in oil. The addition of lecithin (3.5 x 10-5 M) increased significantly the CMC of sodium oleate and stearate (1.1×10^{-4} M and 1.3×10^{-4} M, respectively), probably because lecithin increases the solubility of soaps in oil. The addition of NaCl was observed to slightly decrease the CMC of sodium soaps, probably because increasing salt concentration reduces the electrostatic repulsions between the surfactant head groups, which allows the surfactant molecules to move into closer proximity to each other. Finally, lamellar and cylindrical lecithin RMs were observed to be the most critical structures when filtering oil; the filtering time increased significantly when these types of structures were present. The presence of sodium stearate RMs had no significant effect on the filterability of oil.

Keywords sodium oleate, sodium stearate, calcium stearate, ferric stearate, free-fatty acid, lecithin, salt, reverse micelle, critical micelle concentration, filtration, rapeseed oil, TCNQ solubilization method

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Tiivistelmä

Saippuat ovat amfifiilisiä molekyylejä (surfaktantteja), jotka koostuvat hydrofobisesta ja hydrofiilisestä osasta. Surfaktantit voivat järjestäytyä miselleiksi polaarisessa ympäristössä ja käänteismiselleiksi ei-polaarisessa ympäristössä. Miselli muodostuu kriittisen misellikonsentraation (CMC) yläpuolella. Tehokkaan öljyn puhdistamisen aikaansaamiseksi saippuoiden ja muiden pinta-aktiivisten aineiden käyttäytymisen ymmärtäminen on tärkeää. Tässä diplomityössä tutkittiin neljän eri saippuan (natrium oleaatti, natrium, kalsium ja rauta stearaatti) CMC:tä rapsiöljyssä. Vapaiden rasvahappojen, lesitiinin ja suolan vaikutuksia tutkittin saippuan CMC:hen sekä saippuan vaikutusta lesitiinin CMC:hen. Lisäksi, erilaisten misellirakenteiden vaikutuksia tutkittiin öljyn suodatettavuuteen. CMC määritettiin TCNQ-liukoisuusmenetelmällä. Natrium stearaatin ja oleaatin CMC:t olivat 7,1 x 10^{-5} ja 4,4 x 10^{-5} M. Natrium oleaatin CMC oli hiukan natrium stearaattia matalampi, johtuen todennäköisesti kaksoissidoksesta natrium oleaatin hydrofobisessa osassa. Misellin muodostumista ei havaittu kalsium tai rauta stearaatilla ja siksi jatkotutkimukset tehtiin ainoastaan natrium saippuoille. Jopa pieni määrä vapaita rasvahappoja (5 wt.-%) esti natrium saippuoiden misellin muodostuksen, mikä johtui todennäköisesti siitä, että rasvahappo lisäsi saippuoiden liukoisuutta öljyyn. Lesitiinin lisäys (3,5 x 10^{-5} M) kasvatti natrium oleaatin ja steraatin CMC:tä reilusti (1,1 x 10^{-4} M ja 1,3 x 10^{-4} M), koska on todennäköistä, että lesitiini kasvattaa saippuoiden liukoisuutta öljyyn. Suolan lisäys pienensi natrium saippuoiden CMC:tä, todennäköisesti koska kasvava suolakonsentraatio alentaa elektrostaattisia repulsioita surfaktanttien päiden välillä, mikä mahdollistaa surfaktannttien lähentymisen. Lamellaarisien ja sylinterimäisten lesitiinin käänteismisellien huomattiin vaikuttavan eniten öljyn suodatettavuuteen: suodatusaika tuplaantui näiden läsnä ollessa. Natrium saippuoiden käänteismisellien läsnäolo ei vaikuttanut öljyn suodatettavuuteen merkittävästi.

Avainsanat natrium oleaatti, natrium stearaatti, kalsium stearaatti, rauta stearaatti, vapaa rasvahappo, lesitiini, suola, käänteismiselli, kriittinen misellikonsentraatio, rapsiöljy, TCNQ liukoisuusmenetelmä

Foreword

It has been very exciting to work on this project; I have improved and challenged myself in many areas such as conducting laboratory work, presenting and writing. In addition, I have learned patience and perseverance, which I have noticed to be very helpful with scientific work. I would like to thank my supervisor Monika Österberg for providing me this opportunity to work in this project and for all the good advice you have given me during this project. In addition, I am very grateful to my thesis advisors Robertus Nugroho and Tuula Lehtimaa for your excellent advice and support. Robertus gave me a lot of help in laboratory experiments and broadened my understanding of scientific work, while Tuula helped me to improve my presentation skills and provided me an opportunity to visit Neste's laboratory and oil refinery in Porvoo. I would like to thank this project's coworkers Susanna Kuitunen, Maria Sammalkorpi and Sampsa Vierros for your tips and advice during project meetings. In addition, I would like to assign special thanks to my coworker Nanna Koivula for your endless cheer during this project, it really meant a lot to me to have a colleague like you. Thank you Ritva Kivelä, Marja Kärkkäinen and Rita Hatakka for giving me a lot of practical help in laboratory and thank you Iina Kivimäki for helping me in laboratory experiments during summer. In addition, I would like to thank my boyfriend, friends, parents and fellow students for your encouragement and that you got my thoughts elsewhere during my time off.

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Abbreviation

AOT	Sodium bis(2-ethylhexyl) sulfosuccinate
Ca-stearate	Calcium stearate
CMC	Critical micelle concentration
CMT	Critical micelle temperature
C_{pp}	Geometric packing parameter
Cryo-TEM	Cryogenic transmission electron microscopy
CTAB	Cetyltrimethyl ammonium bromide
DDSa	Dodecenyl succinic acid
DLVO	Derjaguin, Landau, Verwey, and Overbeek
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
FA	Fatty acid
Fe-stearate	Ferric stearate
FFA	Free-fatty acid
HLB	Hydrophilic-lipophilic balance
HPL	Hydratable phospholipid
MLT	Metastable lifetime
NaCl	Sodium chloride
Na-oleate	Sodium oleate
Na-stearate	Sodium stearate
NHPL	Non-hydratable phospolipid
RM	Reverse micelle
RT	Room temperature
SANS	Small-angle neutron scattering
SAXS	Small-angle X-ray scattering
TAG	Triacylglycerol
TCNQ	7,7,8,8-Tetracyanoquinodimethane
o/w	Oil-in-water
w/o	Water-in-oil

1. Introduction

Crude vegetable oils contain various minor substances including impurities such as soaps, trace metals, free-fatty acids (FFA) and phospholipids (Koris and Vatai, 2002; Hamm et al., 2013). These impurities deteriorate the quality of the oil and therefore several steps of oil refining, such as degumming and bleaching are used to remove the minor components. Degumming is usually the first step of crude vegetable oil refining, in which water or dilute acid is used for phospholipid removal (Young et al., 1994; Subramanian and Nakajima, 1997). Generally, bleaching follows the degumming process in the purification process of edible oils and is based on adsorption, which is executed by mixing bleaching clay into oil (O'Brien et al., 2000). Finally, filtration process is used to remove bleaching clay and impurities within (Taylor, 2005).

Soaps are amphiphilic molecules, which are often called surfactants due to their ability to reduce interfacial tension. Surfactants are combinations of both hydrophobic (fat-loving) and hydrophilic (water-loving) parts. (Lombardo et al., 2015) Hydrophobic components have strong tendency to avoid direct contact with water. This thermodynamically unfavorable interaction can be minimized by aggregation of amphiphilic molecules into reverse micelles (RMs) in which the hydrophobic parts are exposed to nonpolar media and the hydrophilic components are shielded. Formation of RMs happens after certain surfactant concentration, which is termed critical micelle concentration (CMC). (Hill et al., 2014) RMs are normally aggregates of 50 to 200 surfactant molecules with the droplet radius of 5-50 nm, whose structure depends on molecular structure, various interactions between molecule and solvent, electrostatics, and homogeneity of the self-assembling components (Lombardo et al., 2015; Hill et al., 2014; Terence, 2010). Generally, spherical micelles are the simplest structure of self-assembled surfactants but also other structures such as lamellar, cylindrical and hexagonal phases are possible (Hill et al., 2014). The understanding of aggregation behavior of soaps and their synergistic effects with other surfactants is crucial for the efficient oil purification.

The aim of this thesis was to determine the CMC of various soaps in nonpolar media and to evaluate how counter-ion valence and presence of additives affect the CMC. The second objective of this thesis was to evaluate how the self-assembly of various micellar systems affect the purification process of oil focusing on filterability. Four different soaps i.e. sodium oleate,

sodium stearate, calcium stearate, and ferric stearate (Na-oleate, Na-stearate, Ca-stearate, Fe-stearate) were investigated in rapeseed oil. The effect of FFAs, lecithin and sodium chloride (NaCl) on the CMC of soaps were studied and the effect of soaps on the CMC of lecithin was investigated.

Section 2 of this work provides a short literature review: theoretical background on natural occurring fats and oils is presented in Chapter 2.1. Impurities present in fats and oils are discussed in Chapter 2.2. Chapter 2.3 provides a short theory behind micelle formation, while Chapter 2.4 deals with self-assembly of amphiphilic compounds in nonpolar media. Chapter 2.5 discusses the effect of citric and phosphoric acid on crystallization behavior of metals. Section 3 presents the experimental part of the thesis, including materials and methods, sample preparation, results and discussion. Finally, section 4 presents the conclusions drawn and discusses future prospects.

2. Literature review

2.1 Natural Fats and Oils

Naturally occurring fats and oils can be of animal or plant origin; when the melting point is above ambient temperature, it is commonly called a fat, and if below, an oil. (Thomas et al. 1987, p.174) Triacylglycerols (TAGs) are the main components of fats and oils (approximately 95-98 %) regardless of their origin. In addition, fats and oils include free fatty acids (FFA) and minor components, which mostly act as impurities. (Kamal-Eldin, 2005, pp. 319–320 and Hirata et al., 2013) Table 1 presents the amount of TAGs, FFAs and some impurities present in crude and refined vegetable oils.

Table 1. The amount of TAGs, FFAs and some impurities present in crude and refined vegetable oils. (Modified from Chaiyasit et al, 2007a)

	Palm		Soybean		Sunflower		Canola	
Components	Crude Oil	refined	Crude Oil	refined	Crude Oil	refined	Crude Oil	refined
Triacylglyserols (%)	93.1	>99	95-97	>99	95.6	>99	-	>99
Free fatty acids (%)	2.0-5.0	<0.10	0.3-7	<0.05	0.8-2.4	<0.05	0.4-1	<0.05
Trace metals								
Iron (ppm)	5.0-10.0	0.12	1.0-3	0.1-0.3	-	-	1.5 -	<0.1
Copper (ppm)	0.05	0.05	0.003-0.005	0.0020.006	-	-	0.1	<0.01
Phosporus (ppm)			510	1				

The basic fat molecule, TAG, consists of a glycerol molecule combined with three molecules of fatty acid (FA) (List, 2009, pp. 1-2). Figure 1 presents the general structure of TAG. Upon hydrolysis, each molecule of TAG can release one glycerol molecule and three FAs (O'Brien et al., 2000, pp. 23). Depending on the number of FAs linked to the glycerol backbone, glycerides can be tri-, di- or monoglycerides (Hernandez and Kamal-Eldin, 2013. pp. 23-24). If all FA molecules are similar, a simple triglyceride is formed and if more than one kind of FA is present, the formed molecule is a mixed triglyceride (List, 2009, pp. 1-2). The TAGs of oils and fats can self-organize into crystal networks based on three polymorphic arrangements, the α-, β-, and β'-TAG polymorphs. The crystal structures of fats and oils are influenced by the FA components of TAG and their positions. In addition, the presence of other lipids can affect the crystal structures of fats and oils in small amounts. The crystal structures of fats and oils determine the solidification and melting points, which are important for some food products such as ice creams and margarines. (Hernandez and Kamal-Eldin, 2013. pp. 23-24)

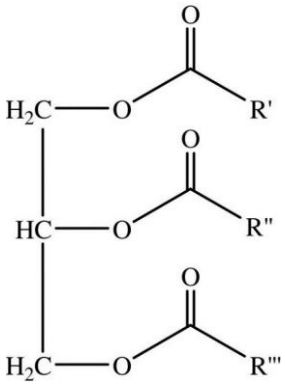


Figure 1. General structure of TAG. R is an alkyl chain that might contain varying amount of double bonds.

FAs are the main component of biological lipids - they are vital constituents of plant and animal tissues. FAs can occur as FFAs or as esterified into glycerol backbone as TAGs. They consist of a carboxylic head group followed by a hydrocarbon chain: the hydrocarbon chain can be unsaturated, saturated, branched, cyclic, oxygenated, nitrogenated, halogenated, or sulfated. Figure 2 represents the general chemical structure of a FA. The most abundant FAs in nature are usually unbranched and monobasic saturated or unsaturated FAs. (Hernandez and Kamal-Eldin, 2013. pp. 18) Even-numbered chain lengths between 4 and 26 carbon atoms dominate in animal and vegetable triglycerides and in waxes chain lengths up to 38 carbon atoms exist. From edible oils and fats, all dominant FAs belong to the families of alkane-

and alkene-FAs. Their nomenclature and structure follow the common rules of chemistry; however, the trivial names are used for the most common FAs. (Bockisch, 1998. pp. 57-61) From edible oils and fats, the main FAs structural differences can occur only in configuration (cis- or trans-) or the position of the double bond. Different kinds of FA structures are for example saturated unbranched FAs, e.g. palmitic and stearic acid, unsaturated unbranched FAs, monounsaturated FAs, e.g. oleic acid, polyunsaturated FAs e.g. Linoleic acid and other FAs. The most common FAs and their occurrence in fats and oils are presented in Table 2. (Bockisch, 1998, pp. 57-61)

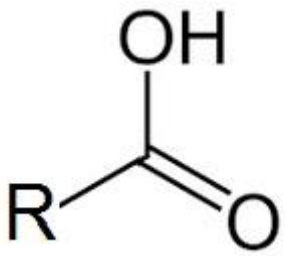
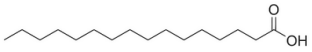
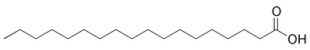
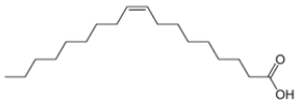
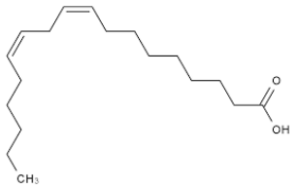


Figure 2. General chemical structure of FAs.

Table 2. The occurrence of common FAs in fats and oils. (Modified from Bockisch, 1998, pp. 64-65)

Trivial name	**Occurrence in common fats and oils (%) of total FAs**
Palmitic acid 	Coconut oil, Palm kernel oil, Sesame oil (7-10) Cottonseed oil (17-25) Milk fat (33-38) Lard (20-30) Tallow (25-40)
Stearic acid 	Lard (16-24) Tallow (15-30)
Oleic acid 	Rapeseed oil (55-65) Peanut oil (45-65) Sesame oil (35-50) Corn oil (40-50) Olive oil (55-85) Goose fat (50-65)
Linolic acid 	Safflower oil (<80) Sunflower oil (<75) Poppy seed oil (<65)

There are three different types of animal fats: rendered fats, butter fats, and fish oils (marine oils) (Bockisch, 1998. pp. 121). Lard, tallow and butterfat are supplied almost completely by three kinds of domesticated animals: pigs, sheep, and cows (O'Brien et al., 2000, pp. 1-2). Rendered fats like lard and tallow are by-products of meat production, while the fat of cow's milk is the main representative of butterfat. Marine oils are mainly by-products of fish caught to be processed as a source of food. Rendered fats, butter fats, and marine oils differ significantly in their FA composition. Marine oils contain huge amounts of polyunsaturated FAs, whereas fats from adipose tissue of pigs, sheep, and cows consist of saturated FAs. (Bockisch, 1998. pp. 121) Rendered fats contain mostly FAs with chain length of 16 or 18 carbon atoms

(Thomas et al., 1987, p. 175), whereas butterfat contain significant amounts of short-chain FAs from chain length of 4 to 10 carbon atoms (Bockisch, 1998. pp. 121).

The seeds of annual plants are the largest source of vegetable oils: almost all plants contain oils or fats in their seeds and the amount varies from very low percentages to as much as 70-80 % (O'Brien et al., 2000, pp. 1-2; Bockisch, 1998. pp. 174). The fruit of oil-bearing trees are the second largest source of vegetable oils, for example, olive, coconut, and palm oils are extracted from the fruit of the oil-bearing tree (O'Brien et al., 2000, pp. 1-2). Most commercial vegetable fats and oils consist mainly of oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids (Thomas et al., 1987, p. 175). Oil fruit and oilseeds contain often by-products, which have to be separated, for example, lecithin in case of soybeans (Bockisch, 1998. pp. 178).

2.2 Impurities present in fats and oils

Many impurities in crude oil such as soaps, phospholipids and FFAs might act as an amphiphilic molecule. Amphiphilic molecules play a significant role as detergents, emulsifiers, dispersants, and foaming and wetting agents in several applications (Rosen, 1989; Holmberg et al., 2002). Surfactants have both lipophilic and hydrophilic components as discussed in the introduction part. In general, the lipophilic part consist of a hydrocarbon chain, while the hydrophilic part can be either ionic or nonionic. There are three different ionic hydrophilic parts: anionic, cationic and zwitterionic. Anionic surfactants have a negatively charged head group, and a positively charged counter ion (such as sodium, or ammonium ions) and the other way around, cationic surfactants have a positively charged head group and a negatively charged counter ion. Zwitterionic surfactants have a head group charged both negatively and positively, while nonionic surfactants do not have charge in the hydrophilic part. (Lombardo et al., 2015) The general structures of surfactants are presented in Figure 3.

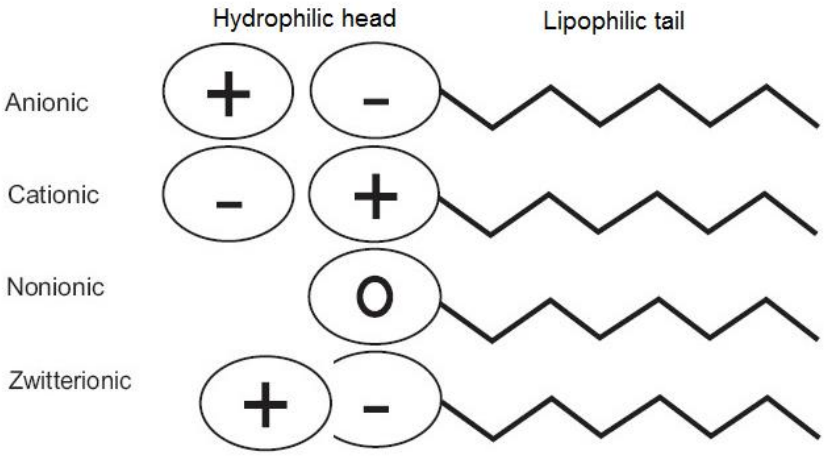


Figure 3. The structures of different surfactants.

Surface-active agents' physicochemical properties and usage are largely determined by their ability to adsorb at an aqueous/organic (oil) or aqueous/air interface. The degree of adsorption is strongly related to the hydrophilic-lipophilic balance (HLB), which is often used to characterize surfactants. HLB means the ratio of the hydrophilic groups' molecular masses to the hydrophobic groups' molecular masses in the molecule. In other words, a high ratio of hydrophilic groups to lipophilic groups indicates a high HLB value and conversely, a high ratio of hydrophobic groups to hydrophilic groups indicates a low HLB value. Hence, compounds with high HLB values (higher than 10) are hydrophilic and compounds with lower HLB values (less than 10) are hydrophobic. Organic molecules might have both polar and nonpolar groups and depending of their relative importance, it may have different properties. For example, if the polar group is ionic and the lipophilic group is a short alkyl chain, the compound is water-soluble and conversely if the compound has several long alkyl chains, it is insoluble in water. (Szymanowski et al., 1984) E.g., oleic acid is hydrophobic and has HLB value around one. Hence, oleic acid is immiscible with water and the molecules are highly lipophilic. On contrast, Na-oleate and Na-stearate have HLB value around 18, which means that they are hydrophilic and miscible with water but almost immiscible in nonpolar media. (Friberg, 1997; Slade, 1998)

Trace metals and metal soaps

Plant oils absorb trace amounts of metals during growing season. These trace metals are harmful to human health, product quality and reduce the efficiency of the oil purification process. The oxidative stability of fats and oils is significantly reduced by trace amounts of iron, copper, manganese, and nickel, whereas sodium, calcium, and magnesium reduce the effectiveness of the degumming, bleaching, and hydrogenation systems. (O'Brien, 2000, pp. 94) When metal ions react with FFA, they form metal salts of FAs (soaps). Soaps with monovalent ion, such as sodium ion, are water soluble, whereas soaps with divalent ion, such as calcium ion, are water insoluble. (O'Brien et al., 2009, p. 525, 536) Water insoluble soaps can form precipitates and they are more difficult to remove from oil (Sen Gupta, 1988). The general structures of sodium and calcium soaps are presented in Figure 4. The exact solubility values of soaps are not reported in literature.

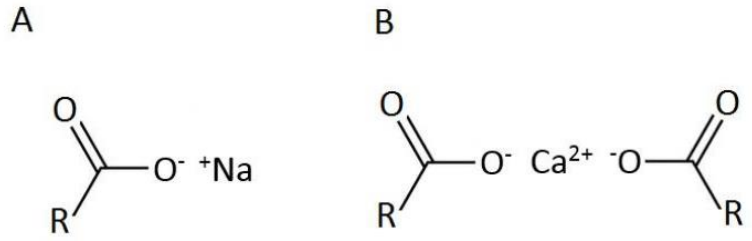


Figure 4. The molecular structure of sodium (a) and calcium (b) soaps.

Metals and metal soaps can be removed by neutralization in chemical refining followed by bleaching in physical refining (Hamm, 2013, pp. 256). However, soap removal by neutralization is preferred since high soap level has been shown to reduce the bleaching efficiency (O'Brien et al., 2009, p. 149). This happens because soaps deactivate the active centers in the bleaching earth (O'Brien et al., 2009, p. 171). In addition, the use of chelating agents can diminish the effects of the metals at different process points, the most commonly used chelating agent is citric acid in degumming phase (O'Brien, 2000, pp. 94).

Moisture and FFAs

Moisture in oils and fats causes hydrolysis, which breaks triglycerides into diglyserides and FFAs such as oleic acid. Fat and oil can come in contact with moisture in many different ways, e.g., an intentional or unintentional addition of water during processing, condensation or broken coils in a vessel. Moisture is removed during processing by vacuum systems that are

utilized with many of the processes. The FFAs cause an unpleasant acidic flavor, which is usually described as rancid; it may also result in a soapy product or in smoke formation during frying. (O'Brien, 2000, pp. 92) Therefore, specified residual level of FFAs after refining is relatively low (0.04–0.10 %). There are two ways to remove FFAs from oil, physical and chemical refining. In physical refining, FFA content is reduced by stripping in a deodorizer, whereas in chemical refining, FFAs are removed by reaction with sodium hydroxide. This reaction forms water soluble sodium soap, which can then be removed by gravity or in a centrifuge. (Hamm, 2013, pp. 253)

Phospolipids

Phospolipids are one of the minor components of crude oils, which are removed at the degumming stage of refining (Hamm, 2013, pp. 24). The solubility of phospholipids affects significantly to the efficiency of the degumming process: they can be divided in two different groups: hydratable (HPL), which are water soluble and non-hydratable phospholipids (NHPL), which tend to remain in oil phase. A typical water degumming process can be enhanced by using acids such as phosphoric and citric acid. The purpose of these acids is to increase the hydratability of phospholipids via chelation and dissociation. (O'Brien, 2009: 8–9) Divalent metal ions such as magnesium and calcium can form a non-hydratable salt with certain phospholipids. Thus, degumming acids are used to liberate the salts from phospholipids. This happens through chelation, in which acids form a binding complex with metal ions. These complexes can then be removed in the water degumming phase. (O'Brien, 2009: 85–86, Oyekunle et al., 2013) With acid degumming, the phospholipid level can be decreased from a typical degumming level 200 µg/g to 20-30 µg/g. (O'Brien, 2009: 8–9)

Pigments

Basically, refined edible oils should be colorless. However, crude vegetable oils have colors varying from red to green. The main reason for these colors are different pigments, which are classified as impurities. The red color is caused by carotenoids and the green color is formed because of chlorophyll. (Hamm, 2013, pp. 256) These pigments are not wanted in refined oils, not only because they produce an undesirable color but also because they may act as sensitizers for photo oxidation (Hamm, 2013, pp. 12). These color pigments are mainly removed during bleaching (Hamm, 2013, pp. 256).

2.3 Self-assembly of amphiphilic molecules – basic theory

For understanding the behavior of surfactants, it is important to understand the theory behind micelle formation and thus, the general micelle theory is discussed in this Chapter. The interfacial forces that occur between particles are discussed briefly in Chapter 2.3.1 and the self-assembly of amphiphilic molecules and the factors affecting on it are discussed in Chapter 2.3.2. In addition, the Winsor's classification of microemulsions are discussed in Chapter 2.3.3.

2.3.1 Interfacial forces between two phases

There are different kind of interfacial forces between particles and molecules that keeps the colloids stable. The main noncovalent interactions and their strengths involved in amphiphiles self-assembly are presented in Table 3. However, in this Chapter, the focus is on understanding the theory named after Derjaguin, Landau, Verwey, and Overbeek (DLVO) focusing on the electrical double layer, since it is the most important concept related to colloidal stability existing in this work.

Table 3. The main noncovalent interaction and their strengths involved in amphiphiles self-assembly. (Modified from Lombardo et al., 2015)

Bonding and interaction type	**kJ/mol**
Ion-ion/ion-dipole/dipole-dipole	200-300/50-200/5-50
Hydrogen bond	4-120
Cation-n(π) interaction	5-80
π-π interaction	0-50
van der Waals interaction	<5
Hydrophobic effects	Entropy
Metal-ligand	0–400

The DLVO theory models the interactions in aqueous colloidal suspensions. It mainly predicts if a colloidal dispersion of charged particles will be stable in aqueous media. (Trefalt and Borkovec, 2014) It is mainly connected with ionic amphiphiles stability and self-assembly in solution (Lombardo et al., 2015). Due to the van der Waals forces, individual atoms can at-

tract each other at short distances for a one-component system (Tadros, 2007). The electrostatic charges on the surface will attract the counter-ions in the electrically neutral aqueous liquid. The counter-ion concentration is higher near the surface than that in the bulk liquid, because of the electrostatic attraction. However, due to the electrical repulsion, the co-ion concentration near the surface is lower than that in the bulk liquid. Therefore, a net charge of excess counter-ions is observed in the region close to the surface, which balances the charge at the solid surface. Therefore, the layer of ions containing the balancing charges and the charged surface is called the electrical double layer. (Li, 2004) Figure 5 presents a schematic picture of an electrical double layer.

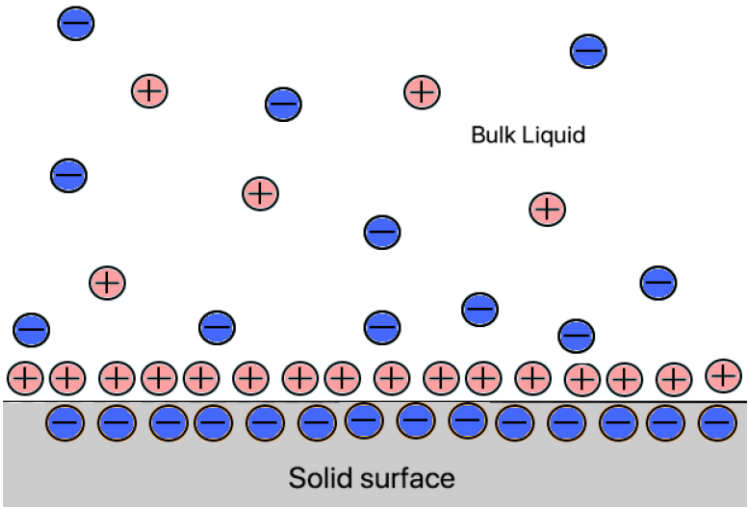


Figure 5. Schematic picture of an electrical double layer.

Fathi et al. (2016) proposed that the average RM size can be defined by the electrical double layer thickness. This thickness is defined by the distance away from a charged surface. This thickness, also known as the Debye screening length, κ^{-1}, is defined in equation (1).

$$\frac{1}{\kappa} = \left(\frac{\varepsilon_r \varepsilon_0 k_B T}{1000 q^2 N_A \sum_i z_i^2 c_{i0}}\right)^{1/2} \quad (1)$$

where ε_r is the relative dielectric permittivity, ε_0 is the electrical permittivity of vacuum, k_B is Boltzmann's constant, T is the temperature, q is the elementary charge, N_A is Avogadro's constant, z_i is the valence of the counter ions, and c_{io} is the counter ion's concentration. (Stokes and Fennell, 1996) At constant temperature for a given system, the electrical double

layer thickness depends only on the valence of ions and counter ion concentration. Considering the system defined by Fathi et al. (2016), equation 1 can be modified to equation (2), which defines the RM size.

$$reverse\ micelle\ size\ \propto \left(\frac{\varepsilon_r \varepsilon_0 k_B T}{1000 q^2 N_A}\right)^{\frac{1}{2}} (z_e{}^2 c_e)^{-1/2} \quad (2)$$

where z_e is the valence of the salt counter ions and c_e is the concentration of the salt counter ions. It can be concluded that RM size depends on at least the valence of the salt counter ion and the concentration of the salt counter ions. It can also be observed that when the valence of counter ion is increased, the RM size is decreased.

2.3.2 Self-assembly of amphiphilic compounds

As discussed in the introduction part, surfactants can self-assembly not only into spherical micelles but also in other self-assembled structures such as lamellar, cylindrical and hexagonal phases. The formed structures depend on parameters such as molecular structure, various interactions between molecule and solvent, electrostatics, and homogeneity of the self-assembling components. (Hill et al., 2014) Simple models of the geometric packing parameter (C_{pp}) can explain different kind of self-assembled structures. C_{pp} expresses the ratio of molecular volume to the surface area of the head groups of the molecule. (Israelachvili et al., 1976) The C_{pp} is defined in equation (3) and the parameters of C_{pp} are demonstrated in Figure 6.

$$C_{pp} = \frac{v}{a_0 l_c} \quad (3)$$

Where v and l_c are hydrophobic alkyl chain's volume and extended length, respectively, and a_0 is surfactant molecule's occupied area at the micellar interface (Israelachvili et al., 1976).

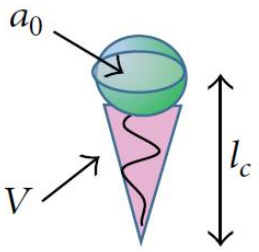


Figure 6. Hydrophobic alkyl chain's volume (V), cross-sectional area (a_0) and extended length (l_c). (Lombardo et al., 2015)

The different kind of micelle structures can be predicted with simple models of the geometric packing parameter as presented in Table 4. Figure 7 presents different structures available both in normal and reverse forms.

Table 4. Predicted micelle structures with with simple models of the geometric packing parameter. (Lombardo et al., 2015; Hill et al., 2014)

C_{pp} value	**Predicted micelle structure**
$0 < C_{pp} < 1/3$	Spherical micelles
$1/3 \leq C_{pp} < 1/2$	Cylindrical micelles
$1/2 < C_{pp} < 1$	Bilayer vesicles
$C_{pp} \approx 1$	Lamellar
$C_{pp} > 1$	Reverse micelles

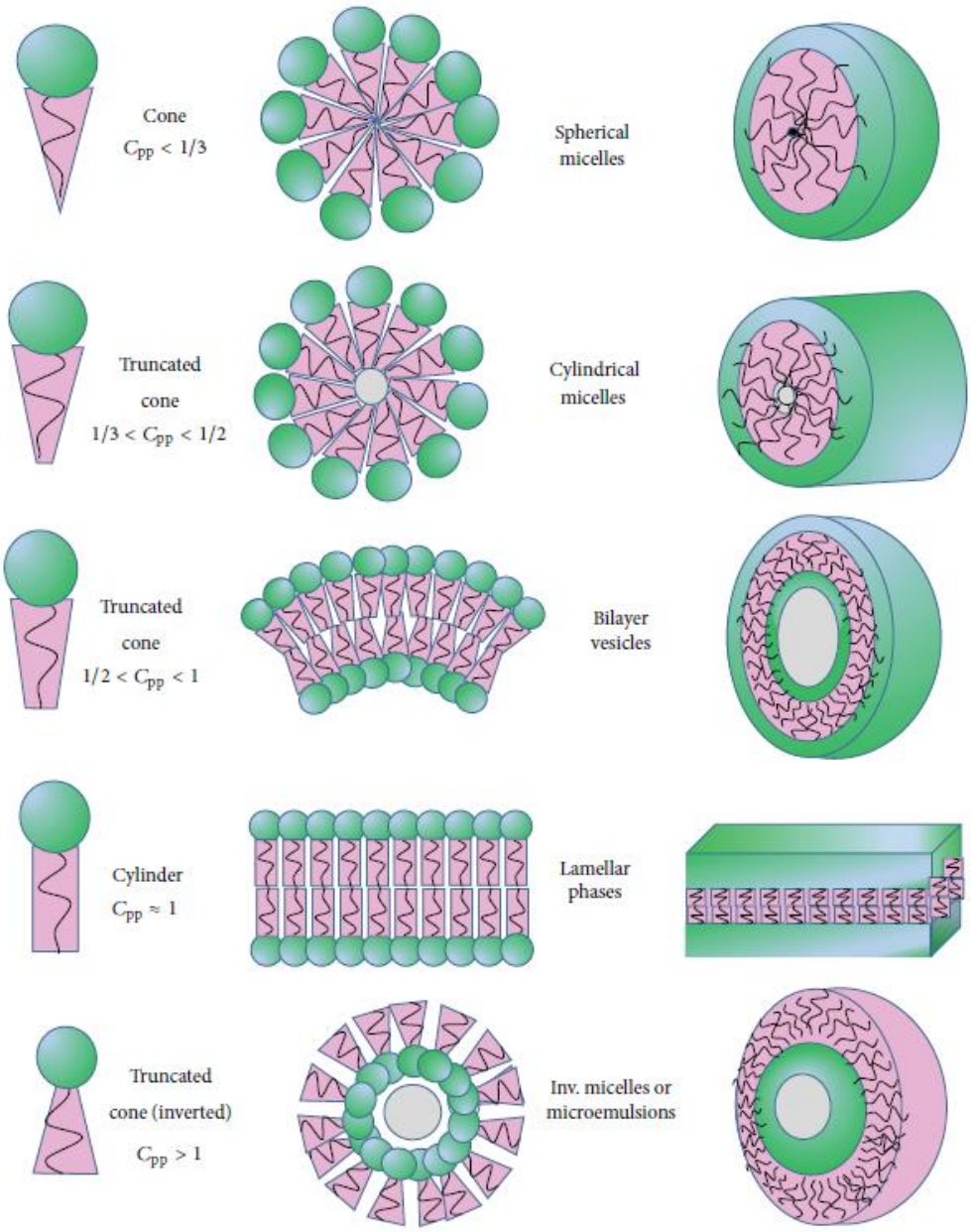


Figure 7. Schematic representation of surfactant self-assembled structures. (Lombardo et al., 2015)

In addition to C_{pp}, water has been considered one of the critical factor when different kind of structures are formed. For example, lecithin is a hygroscopic material that can adsorb water. Various structures are formed depending on the amount of water molecules per lecithin molecule. Lamellar phase dominates in lecithin-water phase diagrams but with lower water amounts (below 10 wt.-%) other structures are formed, such as hexagonal and cubical structures. (Shchipunov, 1997)

Many factors, such as structure of the surfactant, counter-ion nature, presence of additives and change in temperature, are known to have strong effect on the CMC in aqueous media. CMC decreases logarithmically with carbon number of hydrocarbon chain and the relationship usually fits the Klevens equation (4).

$$log_{10}(CMC) = A - Bn_c \quad (4)$$

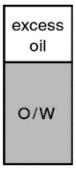
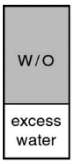
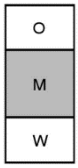
Where n_c is the number of carbon atoms in the chain, $C_nH_{2n+}1$ and A and B are constants for a particular homologous series and temperature. Chain branching gives a higher CMC compared to straight-chain surfactant in aqueous media. On the other hand, surfactants with the same hydrocarbon chain can have different CMC by varying the size or nature of the head group (i.e. from ionic to non-ionic) in aqueous media. For example, for a C_{12} hydrocarbon the CMC with a non-ionic head group lies in the range of 1 x 10^{-4} mol/dm^3, whereas with an ionic head group exhibits a CMC in the range of 1 x 10^{-3} mol/dm^3. Micelle formation is related to the interaction of the ionic head group with the solvent in ionic surfactants. An increase in the degree of ion binding will decrease the CMC, since electrostatic repulsions between ionic groups are greatest for complete ionization. Divalent or trivalent counter-ion valence produces a sharp decrease in the CMC when compared to monovalent valence. The presence of additional electrolytes causes also a decrease in the CMC of most surfactants, because salt screen the electrostatic repulsion between the head groups. The greatest effect is found for ionic materials, whereas non-ionic and zwitterionic surfactants display significantly smaller effect. Finally, the influence of temperature is usually weak and the major effects are the Kraft and cloud points. However, temperature causes a strong effect for polymeric surfactants and thus is common to define a critical micelle temperature (CMT) for this class of surfactants. (Terence, 2010)

2.3.3 Winsor's classification of microemulsions

One of the widest used definition of a microemulsion is from Danielsson and Lindman (1981) "a microemulsion is a system of water, oil and an amphiphile, which is a single optically isotropic and thermodynamically stable liquid solution". Microemulsions can be compared to small-scale versions of emulsions, with a drop's radius size in the range of 5–50 nm. However, there are many differences between microemulsions and emulsions; emulsions are thermodynamically unstable and their formation requires input of work, while microemulsions form spontaneously when the conditions are right. Winsor has classified microemulsions in four

general types (I-IV) of phase equilibria as presented in Table 5. Types I, II, III or IV form depending on sample environment and surfactant type. (Terence, 2010, pp. 91-96)

Table 5. Winsor's classification of microemulsions (O = oil, W = water, M = microemulsion). (Modified from Terence, 2010, pp. 95-96)

Type	Picture	Type of emulsion
I		Surfactant is preferentially soluble in water and (o/w) microemulsion is formed. The (o/w) microemulsion coexist with excess amount of oil.
II		Surfactant is mainly in the oil phase and (w/o) microemulsions is formed. The (w/o) microemulsion coexist with the surfactant-poor aqueous phase.
III		A three-phase system where a surfactant-rich middle-phase (microemulsion) coexists with both excess oil and water surfactant-poor phases
IV		A single-phase (isotropic) microemulsion, that forms upon addition of a sufficient quantity of amphiphilic molecules.

A ratio of R (Winsor's ratio) is used to account the interactions between amphiphilic molecules and solvents on interfacial curvature. Hence, the R-ratio compares the tendency for an amphiphilic molecule to dissolve into oil, to its tendency to disperse in water. Three distinct regions can be recognized in microemulsions: an oil or organic region, O, an aqueous region, W, and an amphiphilic region, C. Interfacial film stability is determined by the C layer, where

cohesive interaction energies exist. The cohesive energy between molecules x and y is defined as A_{xy}. A_{xy} can be considered to be composed of surfactant - oil and surfactant - water interactions as presented in equation (5). (Terence, 2010, pp. 91-99)

$$A_{xy} = A_{Lxy} + A_{Hxy} \quad (5)$$

where A_{Lxy} is interaction between nonpolar parts and A_{Hxy} represents polar interactions of the two molecules, especially hydrogen bonding. Hence, cohesive energies for surfactant - oil and surfactant - water interactions can be written as presented in equations (6) and (7). (Terence, 2010, pp. 91-99)

$$A_{co} = A_{Lco} + A_{Hco} \quad (6)$$

$$A_{cw} = A_{Lcw} + A_{Hcw} \quad (7)$$

Generally, A_{Hco} and A_{Lcw} are very small values and can be ignored. The cohesive energy A_{co} promotes the surfactant molecules miscibility with oil and A_{cw} with water. In summary, Winsor's ratio of cohesive energies, stemming from interaction of the interfacial layer with oil, divided by energies resulting from interactions with water, determines the preferred interfacial curvature. Winsor's ratio can be written as presented in equation (8). (Terence, 2010, pp. 91-99)

$$R = \frac{A_{co}}{A_{cw}} \quad (8)$$

If R > 1 Winsor's type II (w/o) microemulsion is formed and the interface tends to increase its area of contact with oil and if R < 1 Winsor's type I (o/w) microemulsion is formed and the area of contact with water is increased. Moreover, if R = 1 a balanced microemulsion is formed. In addition to surfactant interactions with solvents, other cohesive energies are water - water (A_{ww}), oil - oil (A_{oo}), hydrophobic - hydrophobic parts (L) of surfactant molecules (A_{LL}) and hydrophilic - hydrophilic parts (H) of surfactant molecules (A_{HH}). Figure 8 presents a simplified picture of water and oil interface containing some surfactant molecules and points out the interaction of different components in microemulsion. (Terence, 2010, pp. 91-99)

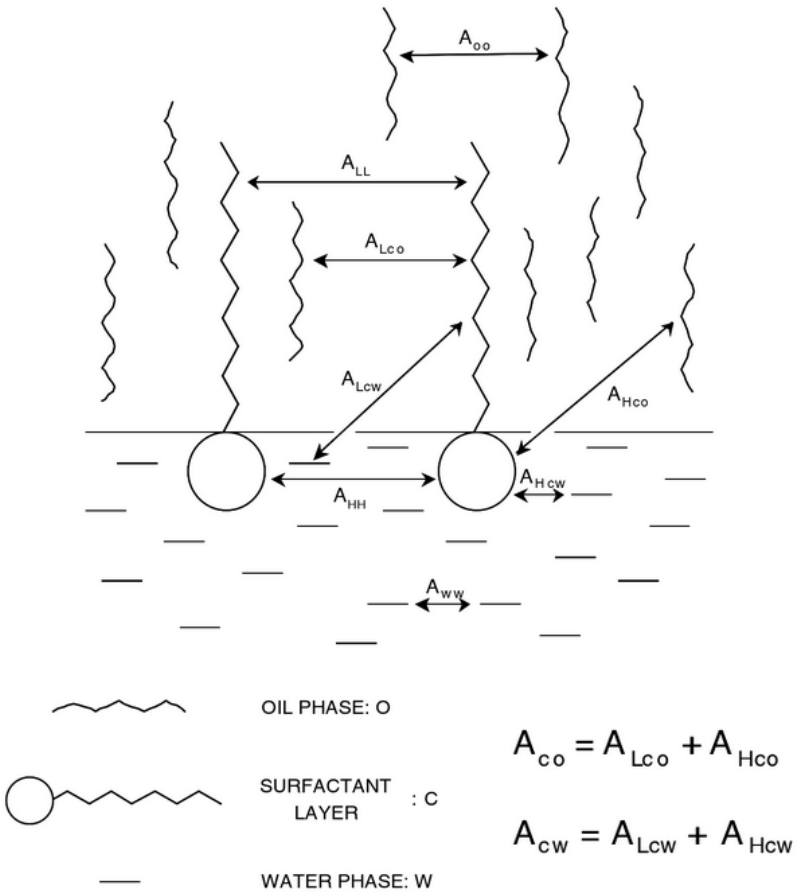


Figure 8. Different interactions in microemulsions. (Modified from Terence, 2010).

2.4 Self-assembly of amphiphilic compounds in nonpolar media

RMs are mostly formed in the ternary mixtures of surfactant/water/oil (Tadros, 2011). Thus, water has been regarded as a necessary component in the formulation of RMs in most studies carried out in the past (Khoshnood and Firoozabadi, 2015; Tadros, 2011, pp. 17). However, few studies describe the formation of RMs in nonpolar media without water addition (Tadros, 2011, pp. 17). Many studies have also shown that the size of a RM is highly dependent on the water-to-surfactant ratio: the size a of RM is increased with increasing water-to-surfactant ratio (Nanni and Dei, 2003; Li et al., 2000; Fang and Yang, 1999).

Chapter 2.4.1 describes the RM formation of soaps. The formation of mixed micelles are discussed in Chapter 2.4.2, e.g. the effect of solvent and other surfactants to the self-assembly of amphiphilic compounds such as lecithin and soaps. In addition, the effect of electrolytes on the self-assembly of amphiphilic compounds is described in Chapter 2.4.3.

2.4.1 Soaps

Monovalent soaps such as sodium soaps and di- and trivalent soaps such as Ca- and Fe-stearate tend to form reverse micellar structures in oil (Sen Gupta, 1988). However, they do not form similar kind of RMs, because of varying counter ion valence, which leads to different number of FA attached to them. The general molecular structures of Na-oleate, Na-stearate, Ca-stearate and Fe-stearate are presented in Figure 9. Monovalent soaps have only one FA, whereas divalent soaps have two and trivalent soaps have three FAs attached. This leads to differences in molecular packing parameter (C_{pp}). The molecular packing parameter for monovalent soap is ca. one and for di- and trivalent soaps it is more than one. Thus, monovalent soaps tend to form lamellar structures and di- and trivalent soaps tend to form RMs as presented in Figure 10. (Eastoe, 2005)

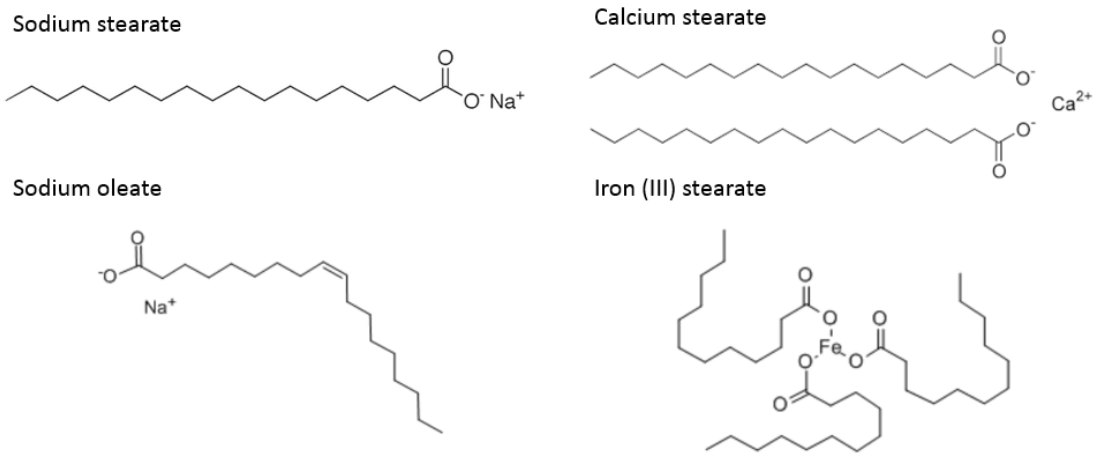


Figure 9. The general molecular structures of Na-stearate, Na-oleate, Ca-stearate and iron (III) stearate.

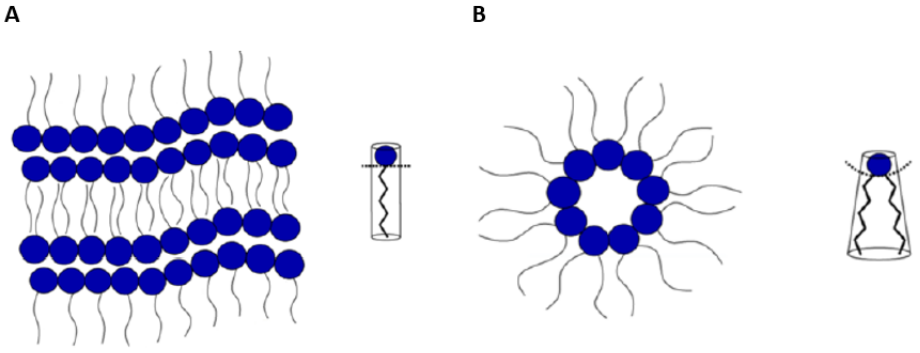


Figure 10. Micellar structures of monovalent soap (a) and divalent soap (b) in oil. (Eastoe, 2005)

2.4.2 Mixed micelles

The concentration of surface-active molecules or changes in lipid medium composition, could lead to exchange of surface-active substances between the medium and RM. This might affect the characteristic and structure of RMs. (Kittipongpittaya et al., 2014) Many studies have shown that the size of RM is increased with increasing water-to-surfactant ratio (Nanni and Dei, 2003; Li et al., 2000; Fang and Yang, 1999). In addition, the size and shape of RM can be altered by the presence of co-surfactants (Kittipongpittaya et al., 2014). For example, Chaiyasit et al. (2007b) studied the sodium bis(2-ethylhexyl) sulfosuccinate (AOT) RM model system and observed that phospholipids caused the RM size to increase while cumene hydroperoxide and oleic acid caused the RM's size to decrease.

Nikofordis and co-workers (2015) observed the same phenomenon with respect to micelle size decrease by oleic acid. They investigated the assembly behavior and gel formation of different compositions of an unsaturated FA, oleic acid, and Na-oleate, in sunflower oil. The aggregation behavior was studied under crossed polarized light at 20 °C and the nanoscale self-assembly of the samples was studied under small-angle neutron scattering (SANS) in decane. It was observed that the aggregate structures of the mixture of oleic acid and Na-oleate, and Na-oleate alone differed completely. Figure 11 presents the photomicrograph of the samples observed under crossed polarized light at 20 °C. Each studied sample contained 16 wt.-% of the filler (oleic acid: Na-oleate) at different ratios. It was found out that Na-oleate formed larger aggregates whereas the mixture of oleic acid and Na-oleate had significantly smaller crystals. No crystal formation was observed for oleic acid alone, as expected.

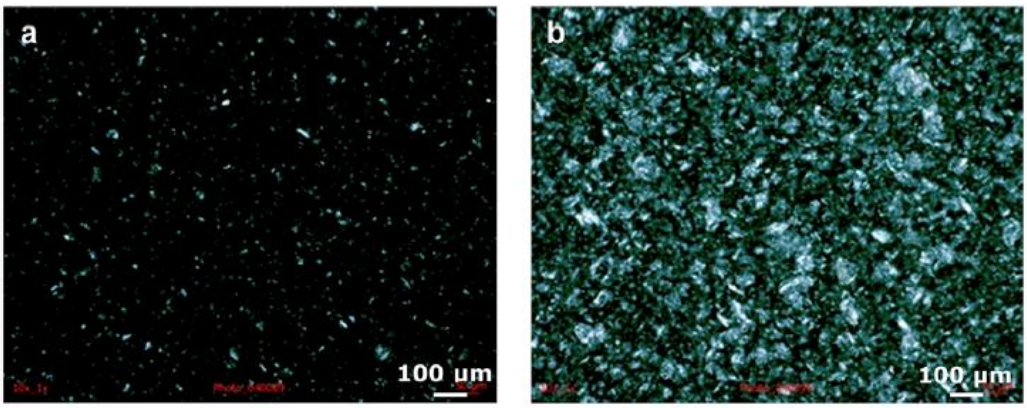


Figure 11. Photomicrograph of organogels observed under crossed polarized light at 20 °C. The oleic acid: Na-oleate ratio in these pictures was 1:1 (a) and 0:1 (b) (Nikiforidis et al., 2015).

Nikifordis et al. (2015) observed that oleic acid inhibits the extensive aggregation of Na-oleate. Figure 12 presents the molecular structures of oleic acid and Na-oleate. Nikifordis et al. (2015) also concluded that mutual assembly is present when oleic acid and Na-oleate are mixed. This was expected, since it has been proven that oleic acid increases the solubility of Na-oleate (Tandon et al., 2001; Guo and Fu, 2000). The strong interactions between the hydrophilic head groups of Na-oleate molecules lead to the formation of lamellar structures and large crystals.

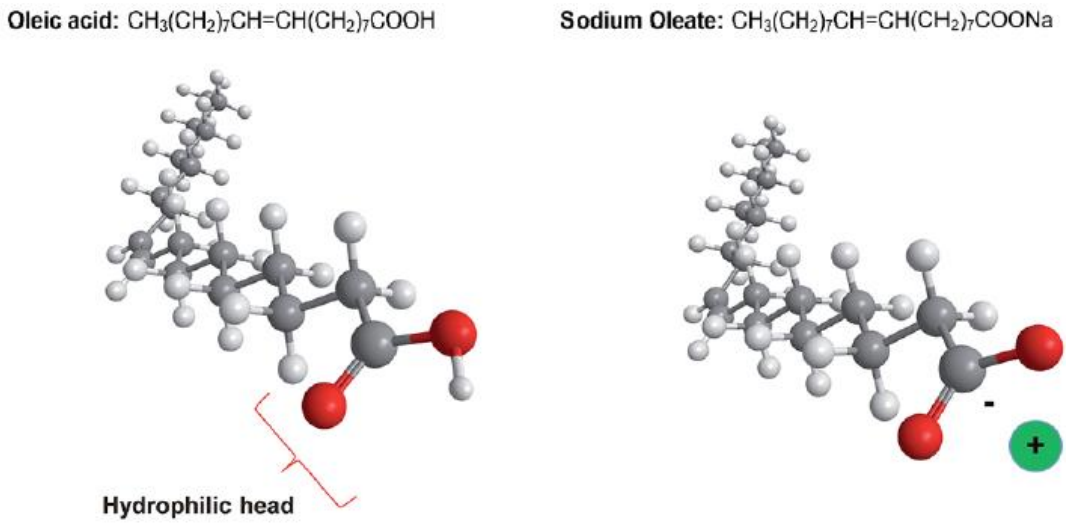


Figure 12. The molecular structures of oleic acid and Na-oleate.

2.4.3 The effect of electrolytes on aggregation

Many studies have shown that increasing ionic concentration causes swelling of micelles in regular (o/w) microemulsions (Porte and Appell, 1981; Missel et al., 1980, 1989; Mazer et al., 1976). It is also observed by many researches that spherical shaped micelles are transformed into ellipsoidal shape when ionic concentration is increased (Alargova et al., 1998a, 1998b). Moreover, salt addition has been observed to decrease the CMC of surfactants in aqueous media as discussed in Chapter 2.3.2. For example, Yu et al. (2010) studied the effects of inorganic and organic salts on aggregation behavior of cationic gemini surfactants in aqueous system. They concluded that salts reduced effectively the CMC values of the cationic gemini, since iomized groups can get closer due to the thinner double layer repulsion.

On the other hand, when ionic concentration is increased in (w/o) microemulsions, the behavior of surfactants is not that unambiguous. Some of the studies have shown that salt addition decreases the size of RMs (Li et al., 2000; Sheu et al., 1986) while others have shown no change (Fang and Yang, 1999; Henle et al., 2007) or even increase (Nagy, 1989) in RM's size. Moreover, there are fewer studies on the effect of increasing ionic concentration on the CMC of surfactants in non-aqueous media compared to the aqueous media. Thus, it is very difficult to find relevant literature concerning the effect of electrolytes on the CMC of surfactants in nonpolar media. Wan and Poon (1969) studied the interfacial tension of liquid paraffin/cetrimide system when salt concentration was increased. They observed that interfacial tension was reduced as the salt concentration was increased. They studied the effect of both anionic and cationic ions on the CMC of cetrimide. The anionic gegenions were more effective in reducing the CMC compared to the cationic ions in the case of cationic surfactant. The effect of anionic gegenions on depression of the CMC of cetrimide was as follows for sodium salts: Br- > C6H5O73- > Cl- > SO42- and for ammonium salts: Br- > Cl- > SO42-. The various cations showed a slight effect on the CMC and the order of effectiveness was as follows: Na+ > NH4+ > K+ and no differences were observed between chlorides and sulfates: Mg2+ = Li+ = NH4+ = Na+ = K+. However, this article is very old and dubious and thus, it is obvious that more research needs to be conducted in this area.

Li et al. (2000) synthesized nanophase zirconia particles by mixing two RM solutions, which contained salt zirconyl chloride ($ZrOCl_2$) and base ammonium hydroxide (NH_4OH). The RM structure was characterized using Small-angle neutron scattering (SANS) at room temperature (RT). It was found out that with the addition of the base and salt, the RM size decreased compared to the RMs without salt and base addition. It was also observed that these RMs exhibit spherical shape and uniform size. While Nagy (1989) studied the (cetyltrimethyl ammonium bromide (CTAB))-1-hexanol-water microemulsion in the presence of Co (II) and Ni (II) ions. The size of micellar aggregates was studied using Fluorine-19 nuclear magnetic resonance (F-NMR). It was observed that the size of micellar aggregates increased with increasing Co (II) and Ni (II) concentrations and with increasing water content.

In addition, Fang and Yang (1999) synthesized ZrO_2-$Y2O_3$ nanoparticles by using CTAB/hexanol/water RMs and investigated the relationship between the micelle microstructure and size, morphology, and aggregate properties of particles prepared. It was found out that high CTAB concentration ([CTAB] > 0.8 mol/l) did not affect the microstructure of RM. However,

high CTAB concentration with high water and metallic ion concentration was observed to increase the RM droplet size. These micelles were found out to be spherical in shape.

Fathi et al. (2012) wanted to verify the phenomena of salt addition to the RM structure and stability. They investigated the RM stability and structure with the addition of NH_4OH, $ZrOCl_2$, and $Al(NO_3)_3$ salts. They used dynamic light scattering for characterization. It was observed that salt additions cause decrease in average RM size. The electrical double layers become thinner as salt concentration is increased, which allows the charged groups to move closer proximity to each other. Gradually, this phenomenon leads to destabilization of the micelles.

2.5 The effect of citric and phosphoric acid on crystallization behavior of metals

Protonation of soaps removes metal ions from soaps creating oil-soluble fatty-acids and free metal ions. Protonation happens at low pH and thus, acids activate the protonation reaction of soaps. (Soontravanich, 2007, pp. 122–158) These metal ions may have an effect on the filtration of oil. Microemulsion solutions (w/o) have nano-sized water pools that are dispersed in the continuous oil phase and stabilized by surfactant molecules at the water/oil interface. These surfactant-surrounded water droplets offer a microenvironment for the formation of nanoparticles. With this kind of technique, ultrafine metal particles can be prepared within the size range 5 nm < particle diameter < 50 nm. The particles are formed when microemulsion is mixed with an aqueous solution carrying the appropriate reactants in order to form the desired particles. The interchange of the reactants occurs when collision of the water droplets takes place in microemulsions. Hence, the nucleation and growth of the metal particles occurs inside the water pools, which control the final size of the particles. (Capek, 2014)

Fats and oils can also naturally contain minor components such as trace metals, as discussed before. Sodium is a trace metal, which can occur for example as counter ions, soaps or as salts when it reacts with FFAs in fats and oils. In addition, refining processes, such as neutralization of the FAs with sodium hydroxide and hydration of the phospholipids with assistance of phosphoric or citric acid, can increase the amount of sodium and acids in lipid environment, respectively. Citric acid is a carboxylic acid, which can form metal carboxylate salts for example through a reaction with sodium hydroxide (Lacey et al., 2012). The reaction mechanism of citric acid reacting with sodium hydroxide is presented in Figure 13. The reaction product is trisodium citrate as seen below.

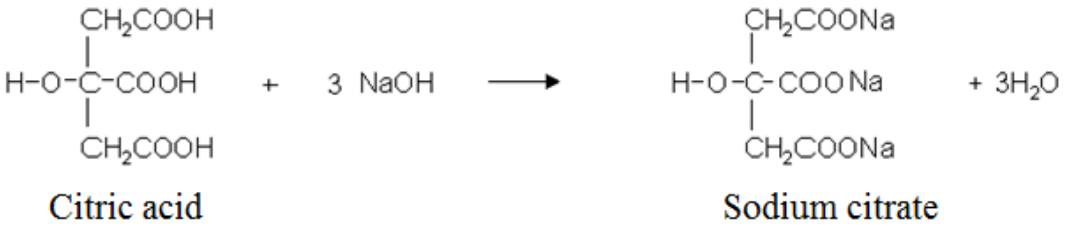


Figure 13. The reaction mechanism for the preparation of sodium citrate.

Lacey et al. (2012) demostrated the reaction of sodium hydroxide and carboxylic acid, dodecenyl succinic acid (DDSa), to form metal carboxylate salts. The reaction was performed at low temperatures (40 to 43°C) in non-aqueous media that contained diesel fuel containing 5 vol.-% FA methyl ester (FAME). The final composition of DDSa and sodium were approximately 800 and 474 mg/kg, respectively. The visible precipitate was observed in less than a minute. In addition, increasing temperature was observed to accelerate the salting reaction. They performed experiments in full-scale and laboratory-scale. In full-scale experiments, diesel was mixed with 300 mg/kg of Na-oleate. It was observed that no deposit was formed under a critical temperature of approximately 150°C. In laboratory-scale experiments, 1 ml of DDSa was added to 10 ml of diesel fuel containing 4.5 ml of sodium hydroxide in ethanol (33000ppm of DDSa and 805ppm of Na). This solution was heated in different temperatures, 120, 140, 150, 160 and 180°C, for four hours. It was observed that the deposit starts to form approximately at 120°C and accelerates beyond 150°C. Figure 14 presents the deposit formation with increasing temperature. It is noteworthy, that the deposit thickness values are approximate. It was also observed that deposits form in pressure release areas with association of high temperature.

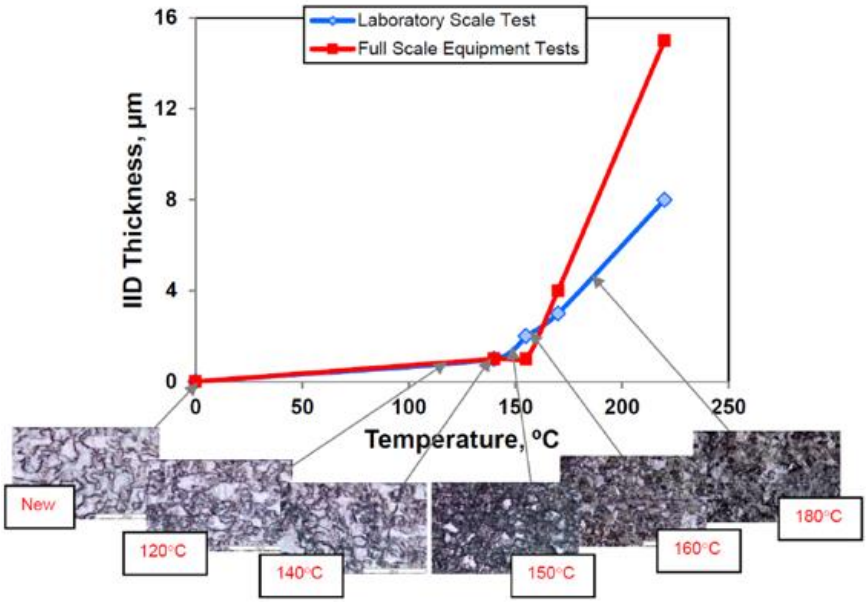


Figure 14. The effect of temperature on the carboxylate salt deposit formation. (Lacey et al., 2012)

On the other hand, nucleation of crystals from aqueous solution is a highly complex process. It includes dehydration of ions, approach of like charges and arrangement of the constituent molecules or ions into an ordered 3-dimensional structure. (Kashchiev and Van Rosmalen, 2003) In contrast, the formation of amorphous phases have significantly fewer constraints (Vekilov, 2010) and thus, it is reasonable that some crystals precipitate from solution via amorphous phases (Wang et al., 2012).

Saha et al. (2012) investigated the early stages of particle formation during the hydration of calcium sulfate hemihydrate ($CaSO_4 \cdot 0.5\ H_2O$) to gypsum crystals ($CaSO_4 \cdot 2\ H_2O$). They used time-resolved cryogenic transmission electron microscopy (TR-cryo-TEM) on a supersaturated solution of calcium sulfate hemihydrate. They observed nanoscale amorphous clusters that grew into amorphous particles and then reorganized to crystalline gypsum within tens of seconds, as hydration proceeds. This multistep model is illustrated in Figure 15. Particles with sizes of 2-4 nm were observed after 10 seconds of sample preparation, which proves that gypsum crystal evolve from nanoscale particles.

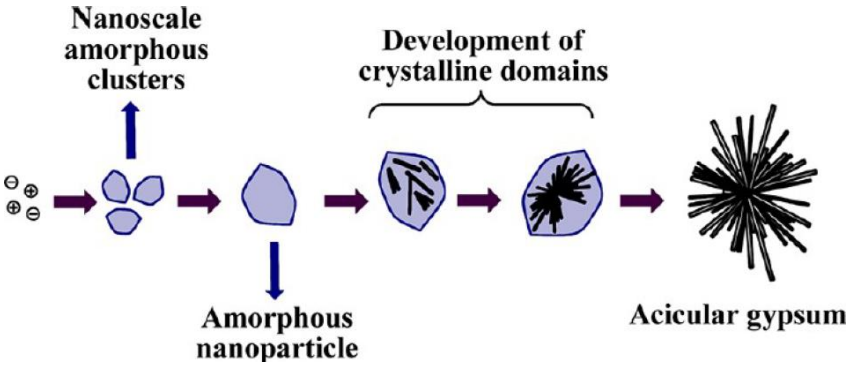


Figure 15. The early stages of gypsum formation illustrated as multistep model. (Saha et al., 2012)

Wang et al. (2012) confirmed the same phenomenon. They investigated the early stages of calcium sulfate precipitation from aqueous solution at RT. They showed also that amorphous calcium sulfate hemihydrate and calcium sulfate are precipitated prior to gypsum. The addition of a small amount of citric acid was observed to significantly delay the formation of gypsum crystals. They concluded that available calcium ions form complexes with the citric acid by binding to the carboxylic groups. These complexes adsorbs onto a growing particle, which introduces a disorder and delays the crystallization process. This prolongs the time in which the amorphous phase exists. It was observed that the effect of citric acid on delaying crystallization persists up to 24 h in this system but eventually the energetically favorable crystalline phase evolves. It is claimed that this multistep model describes the gypsum crystallization more accurately than classical nucleation theory. The classical nucleation theory predicts that the earliest particles have the same crystal structure than the end product.

The same phenomena of a strong retarding effect of carboxylic acids, especially citric acid, on the spontaneous nucleation and crystal growth during calcium sulfate multi-phases crystallization is also observed by other researches (Prisciandaro et al., 2003; Hill and Plank, 2004). It is observed that even a trace amount of citric acid chelating with metal cations (Misra, 1996; Lee and Reeder, 2006) or adsorbing on the active sites of specific crystal facets (Bosbach and Hochella, 1996) could lead to delay of crystallization. Furthermore, Mao et al., (2014) investigated the effect of citric acid and temperature on the metastable lifetime (MLT) of alpha-calcium hemihydrate. They observed that alpha-hemihydrate exhibits an increase in MLT from 0.5 to 48 h along with temperature from 25 to 65◦C in the concentrated 3.54 M $CaCl_2$solution. They also observed that 0.1–1.0 mM of citric acid allows a redouble expansion of the MLT, especially at temperature from 55 to 75◦C. The chemical adsorption of citric groups on

the crystal surfaces delays the crystallization of new phases, which increases the energy barrier for alpha-hemihydrate. This results in an expansion of MLT.

On the other hand, Dabbs et al. (2005) showed that citric acid inhibits the growth of particles. They investigated the effect of citric acid on the formation of aluminum oxyhydroxide precipitation with dynamic light scattering. They observed that citric acid stabilized both particles and soluble aluminum oxyhydroxides in the respective suspension solution. In suspensions, the addition of citric acid limits or prevents the particle growth and in solutions of aluminum cations, citric acid delays the nucleation. In addition, Violante and Huang (1985) studied the aluminum hydroxide and oxyhydroxide formation at RT with an initial Al concentration of 2×10^{-3} M, at pH 8.2, and at varying concentrations of inorganic and organic ligands commonly found in nature. They observed that ligands promoted the formation of non-crystalline products over crystalline $Al(OH)_3$ polymorphs in the following order: phthalate ~ succinate < glutamate < aspartate < oxalate < silicate ~ fluoride < phosphate < salicylate malate < tannate < citrate < tartrate. Crystalline products were inhibited above critical ligand/Al ratios: the lowest molar ratio at which the production of oxyhydroxides or Al hydroxides was inhibited ranged from 0.02 to 15. Above this critical ratio, ligands coprecipitated with non-crystalline products and remained unchanged for at least five months.

Hsu (1979) also investigated the effect of inorganic ligands on the crystallization of gibbsite from OH-Al solutions. Varying concentrations of Na_2SiO_3 and NaH_2PO_4 were added to a hydroxyl-aluminum solution (0.02 M in Al; NaOH/Al mole ratio =2.6) and diluting to 0.002 M in Al for sample preparation. He found out that phosphate inhibited the crystallization of gibbsite above H_2PO_4/Al ratio of 0.1: no crystalline $Al(OH)_3$ was observed for up to three years when carrying out this experiment. The only reaction products observed were aluminum phosphate. Hsu observed that inorganic ligands inhibited the crystallization of Al-hydroxide in next order: phosphate > silicate > sulfate > chloride > nitrate > perchlorate. Gálvez et al. (1999) observed the same phenomenon: they studied the effect of phosphate on the crystallization of ferrihydrate, which was prepared by hydrolysis of $Fe(NO_3)_3$ solutions. Crystallization was studied at different temperatures (298, 323, and 373 K), pH (3-9) and initial P/Fe atomic ratios. In general, it was observed that the crystallization was inhibited or only weakly crystallized lepidocrocite was formed at P/Fe > 2.5%. In addition, it was found out that the formation of hematite over goethite was favored at all temperatures, for most of the pH and P/Fe ranges investigated in the presence of phosphate.

2.6 Summary

TAGs are the main constituents of fats and oils regardless of their origin. In addition, fats and oils include FFAs and minor components such as soaps, trace metals and phospholipids. Many impurities present in fats and oils act as a surfactant, which allows the RM formation in non-polar media. HBL is an important tool for characterizing surfactants; a high ratio of hydrophilic groups to lipophilic groups indicates a high HLB value, which means that the surfactant is hydrophilic. The other way around, a high ratio of hydrophobic groups to hydrophilic groups indicates a low HLB value and hydrophobic surfactant. Surfactants can self-assembly in various different structures depending on e.g. molecular structure, various interactions between molecule and solvent, electrostatics, and homogeneity of the self-assembling components. C_{pp} is commonly used to predict the different self-assembled structures. In addition, Winsor's ratio is used to account the interactions between amphiphilic molecules and solvents. Hence, the R-ratio compares the tendency for an amphiphilic molecule to dissolve into oil, to its tendency to disperse in water. If the ratio is more than one (w/o) microemulsion is formed and if the ratio is less than one (o/w) microemulsion is formed. Structure of the surfactant, counter-ion nature, presence of additives and change in temperature are known to affect the CMC of surfactants in aqueous media. However, the CMC of surfactants and their synergistic effects with other components in nonpolar media is studied with less extent and thus more research needs to be executed. It is proposed that monovalent soaps form lamellar structures and di- and trivalent soaps form spherical RMs in oil. In addition, it has been proven that oleic acid increases the solubility of Na-oleate in oil and thus, inhibits the extensive aggregation of Na-oleate. Moreover, it has been proposed that increasing electrolyte addition would decrease the CMC of a surfactant in oil and decrease the average size of RM. When soaps react with acids, metal ions are formed, which may have an effect on filterability of oil. It has been studied that the crystallization of metals happens via amorphous phases in nano-sized water pools inside a RM. It has been shown that citric acid delays or inhibits the formation of metal crystals, while phosphoric acid only inhibits the formation of metal crystals. The understanding of aggregation behavior of soaps and their synergistic effects with other surfactants is crucial for the efficient oil purification. Thus, the purpose of the experimental part is to investigate the CMC of four different soaps (Na-oleate, Na-stearate, Ca-stearate and Fe-stearate) in rapeseed oil. In addition, the effect of FFAs, lecithin and salts on the CMC of soaps and the effect of soaps on the CMC of lecithin are studied. Moreover, the effect of various micellar structures on filterability of oil is investigated.

3. Experimental work

3.1 Materials and methods

3.1.1 Materials

Na-oleate, Na-stearate, Fe-stearate and Ca-stearate were purchased from Tokyo Chemical Industry (Tokyo, Japan). Food grade rapeseed oil (K-brand, Finland) was purchased from a supermarket and oleic acid was from VWR Chemicals (Radnor, Pennsylvania, USA). Granular L-alpha lecithin was obtained from Acros Organics (New Jersey, USA) and NaCl was purchased from Oy FF-Chemicals Ab (Haukipudas, Finland). 7,7,8,8-tetracynoquinodimethane (TCNQ) was from Sigma-Aldrich Co., (Missouri, USA). MilliQ water was obtained from Millipore SAS (Molsheim, France). Rapeseed oil contained 96.9 wt.-% triglycerides, 0.6 wt.-% FFAs and less than 0.6-ppm phosphorus, while oleic acid consisted of 84.5 wt.-% oleic acid, 12.5 wt.-% linoleic acid, and 3 wt.-% other FAs, mainly palmitic acid and stearic acid. Lecithin contained approximately 23 wt.-% phosphatidylcholine, 20 wt.-% phosphatidylethanolamine, 14 wt.-% phosphatidylinositol, 8 wt.-% phosphatidic acid, 8 wt.-% minor phospholipids, 8 wt.-% sugars, 15 wt.-% glycolipids, 3 wt.-% triglycerides and 1 wt.-% water.

3.1.2 Drying of the materials

Rapeseed oil and oleic acid were dried using 3Å molecular sieves from Sigma-Aldrich (Missouri, USA). The molecular sieves were dried overnight (20 h) at 175˚C, prior to use. After drying, they were added straight to the solvent bottle. The amount of sieves used for rapeseed oil was 12.5 g for 430 g of oil and for the oleic acid 2.45 g for 89 g of oleic acid. After addition of molecular sieves, the rapeseed oil was dried overnight in heat cabinet at 40˚C in order to enhance drying and reduce the viscosity, while oleic acid was dried overnight at RT. The soaps and lecithin were dried overnight in the desiccator prior to use; in case of lecithin, warm regenerated silica granules were used. The granules were dried overnight in an oven at 105˚C. Warm granules (75 g) were placed into a vacuum desiccator with 5 g of lecithin on an open petri dish.

3.1.3 Determination of moisture contents

The moisture content of the soaps were determined using gravimetric method at 105˚C. The moisture contents of all lipid materials: oil, oleic acid and lecithin were determined using Karl Fischer titration at RT (Mettler-Toledo Titrator DL35). Lecithin was dissolved in chloroform

before moisture content measurements. Three repetitions were made with every sample to ensure the reliability of the results. The average value of these results was calculated and standard deviation was used as an error estimate. For lipid materials, the moisture content was measured before and after drying to ensure the effectiveness of the drying procedure.

3.1.4 Preparation of stock solutions

Stock solutions consisting of four different soaps, i.e. Na-oleate, Na-stearate, Ca-stearate, and Fe-stearate, were prepared by solubilizing these soaps in rapeseed oil and in rapeseed oil: oleic acid mixture overnight at 95 °C under mechanical stirring. The prepared stock solutions in rapeseed oil had soap concentrations in the range of 0.015-0.05 wt.-%, while stock solutions in rapeseed oil: oleic acid mixture had soap concentrations varying between 0.015-15 wt.-%. It is noteworthy, that 0.02 wt.-% of Ca-stearate and 0.05 wt.-% of Fe-stearate could be solubilized in rapeseed oil, while only 0.015 wt.-% of sodium soaps could be solubilized. Lecithin stock solution was prepared by solubilizing 4 wt.-% of lecithin in the rapeseed oil for 3 hours at 70 °C. In addition, four different concentrations of salt stock solutions, 0.1, 0.5, 1 and 100 mM, were prepared by solubilizing pre-determined amount of NaCl with MilliQ water.

3.1.5 Determination of critical micelle concentration

The CMC of various soaps was determined using TCNQ solubilization method (Kanamoto et al., 1981). Samples were prepared using dried solvent and the prepared stock solutions. First, the appropriate amount of soap stock solution was weighed into sample vials and dried solvent was subsequently added. For examining the effect of lecithin to the CMC of soaps in rapeseed oil, certain amount of lecithin was added into several samples with different range of soap concentrations. In contrast, to investigate the effect of soaps to the CMC of lecithin in rapeseed oil, a certain amount of soaps was added to the samples with different concentrations range of lecithin. In addition, for studying the effect of salts to the CMC of soaps in rapeseed oil, 0.005 g of aqueous NaCl (0 - 100 mM) was added into the samples by using a syringe (Hamilton, Sigma-Aldrich, Missouri, USA). The salt-consisting samples were later sonicated for 3 min (Branson Digital Sonifier, Emerson Electric, Co., St. Louis, USA). TCNQ (1 mg/g of sample) was added to each sample. After sample preparation, the samples were agitated in heat cabinet rotator at RT for 5 h. Na-stearate, Ca-stearate, and Fe-stearate became solid at high concentrations at RT, when they were solubilized in rapeseed oil: oleic acid 1:1 mixture, thus they were agitated in heat cabinet rotator at 70°C for 5 h. However, this did not

have an effect on the results. Na-oleate stayed in its liquid form when solubilized in rapeseed oil: oleic acid 1:1 mixture, so it was agitated in heat cabinet rotator at RT for 5 h. After mechanical stirring, the samples were centrifuged with 800 g for 20 min (Thermo Scientific SL 40 FR) to remove the excess TCNQ.

The absorbances were measured using UV spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at wavelength of 480 nm. The samples were measured in quartz cuvettes. The CMC was determined by plotting the absorbance (A) against the soap concentration (c) expressed in logarithmic scale. The CMC can be seen as a turning point in the curve, as drastic increase in absorbance can be observed. Two straight lines are fitted to the data points and the CMC is taken as the x-value (concentration) of the intersection of the lines. Two linear fittings are made for CMC determination and standard deviation is used as an error estimate. This point can also be observed as a color change in the samples as TCNQ reacts with the surfactants. Formed TCNQ anions solubilize in the aqueous micelle cores, which produces a darker color after the CMC value. The experiments were repeated at least twice for ensuring the repeatability of the results.

3.1.6. Determination of the sample's filterability

Nine different samples (400 g per sample) were prepared for filtration experiments as presented in Table 6. The idea of filtration experiments was to observe how different micelle structures affect the filterability of oil, hence lecithin samples were mainly chosen since the micelle structures of lecithin are known from previous studies (Lehtinen et al., 2017). 150 g of the oil sample was filtered through a filter aid pre-coat. The amount of filtered oil was followed as a function of time.

Table 6. Filtration samples. Lehtinen et al. (2017) determined lecithin micelle structures and soap micelles are expected structures.

	Sample	Expected micelle structure	Lecithin (mol/l)	Sodium stearate (mol/l)	Oleic acid (mol/l)	Water (mol/l)
1	Reference (Rapeseed oil)	No micelles	-	-	-	7.6×10^{-3}
2	Lecithin below CMC	No micelles	1.7×10^{-4}	-	-	7.6×10^{-3}
3	Lecithin above CMC	Cylindrical lecithin RMs	1.2×10^{-2}	-	-	7.6 x 10-3
4	Lecithin above CMC (high water content)	Lamellar	1.2×10^{-2}	-	-	1.1×10^{-1}
5	Lecithin above CMC + 5 wt.-% oleic acid	Lamellar	1.2×10^{-2}	-	45.4	7.6×10^{-3}
6	Lecithin above CMC + 15 wt.-% oleic acid	No micelles	1.2×10^{-2}	-	136.2	7.6×10^{-3}
7	Na-stearate below CMC	No micelles	-	9.4×10^{-6}	-	7.6×10^{-3}
8	Na-stearate above CMC	RMs	-	4.5×10^{-4}	-	7.6×10^{-3}
9	Na-stearate above CMC (high water content)	RMs		4.4×10^{-4}	-	1.1×10^{-1}

3.2 Results and discussion

3.2.1 Moisture contents

The moisture contents of the soaps, determined by gravimetric method, are presented in Table 7. Table 8 presents the moisture contents of the lipid materials, determined by Karl Fischer titration method. As it can be seen from Table 8, the water content reduced for each of the components, so the drying process of the lipid materials succeeded. The drying was slightly more effective to rapeseed oil compared to oleic acid. It can also be observed that lecithin contained more water than the solvents, which is logical since lecithin is a very hygroscopic material. In addition, more water was left in lecithin compared to the solvents, which is probably due to the different drying methods. In case of solvents, molecular sieves were in direct contact with the solvent whereas lecithin was not in direct contact with silica granules. It is also possible, that the affinities of silica and molecular sieves towards water are different and thus, more water was left in the lecithin compared to solvents.

Table 7. The moisture content of the soaps determined by gravimetric method.

Soap	Moisture content (wt.-%)
Na-stearate	1.5 ± 0.008
Na-oleate	0.23 ± 0
Fe-stearate	1.5 ± 0.01
Ca-stearate	2.3 ± 0.03

Table 8. The moisture content of lipid materials determined by Karl Fischer titration method.

Component	Moisture before drying (wt.-%)	Moisture after drying (wt.-%)
Rapeseed oil	0.04 ± 0.012	0.015 ± 0.002
Oleic acid	0.036 ± 0.008	0.028 ± 0.001
Lecithin	0.930 ± 0.006	0.397 ± 0.004

3.2.2 The critical micelle concentrations of soaps in rapeseed oil

The CMC of various soaps in rapeseed oil were determined using TCNQ-solubilization method at RT. Typical TCNQ colour changes, from yellowish to greenish, were observed with Na-oleate and Na-stearate (Figure 16a and Figure 16b). On the contrary, no colour changes from yellowish to greenish were observed for Ca-stearate and Fe-stearate at any concentrations (Figure 16c and Figure 16d). A faint color change from yellowish to more orange could be seen in the ferric-stearate sample series; however, it is caused by ferric-stearate itself, as it has strong red color and it was observed immediately after mixing soap stock solution and pure oil. The CMC values for various soaps are presented in Table 9. The exact soap concentrations in samples are presented in Appendix 1. The graphs showing absorbance as a function of soap concentration are presented in Figure 17. The experiments were repeated twice, and the results were reproducible.

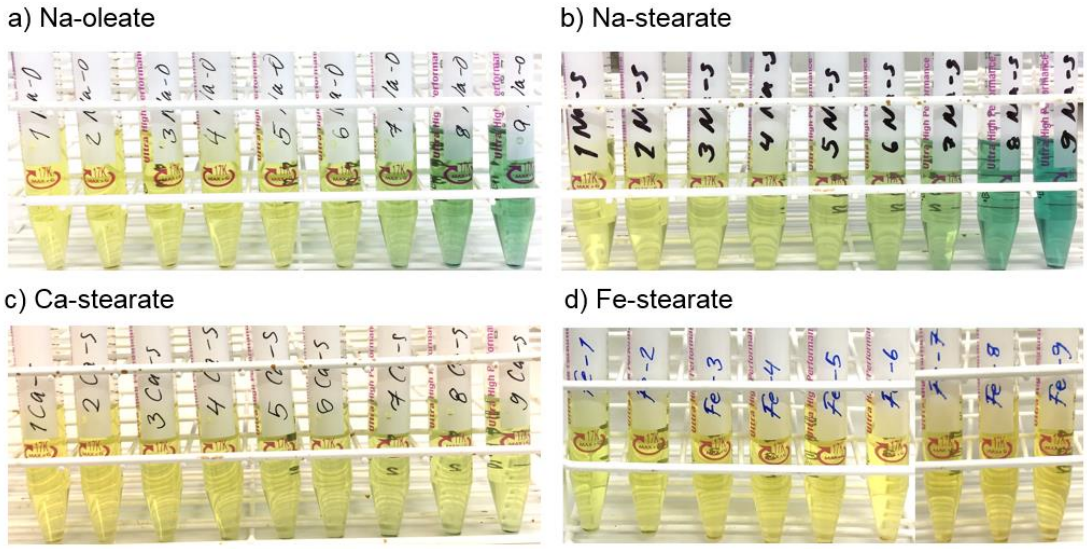


Figure 16. Photographs of the samples after TCNQ addition and centrifugation at various soap concentrations (left to right: lower to higher concentration).

Table 9. The CMC of various soaps.

Soap	**CMC (mol/l) at RT**
Na-oleate	$4.4 \times 10^{-5} \pm 1.2 \times 10^{-5}$
Na-stearate	$7.1 \times 10^{-5} \pm 2.0 \times 10^{-5}$
Fe-stearate	N/A
Ca-stearate	N/A

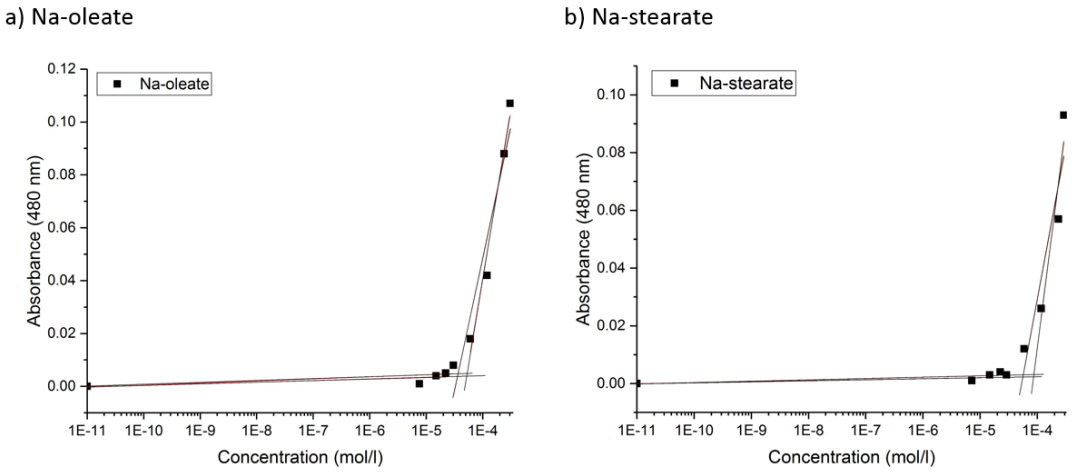


Figure 17. The absorbance of TCNQ as a function of soap concentration at RT.

According to the TCNQ experiments, Na-oleate and Na-stearate are forming RMs in rapeseed oil. The CMC of Na-oleate, $4.4 \times 10^{-5} \pm 1.2 \times 10^{-5}$ M, was slightly lower than the CMC of Na-stearate, $7.1 \times 10^{-5} \pm 2.0 \times 10^{-5}$ M, which means that Na-oleate forms micelles more easily. Since the HLB value of Na-oleate and Na-stearate is about the same (around 18) (Friberg, 1997; Slade, 1998), they have as many carbons in hydrocarbon chain and the head groups of the soaps are similar, the difference in the CMCs result from the double bond in the hydrocarbon chain of Na-oleate. It is known that C = C double bond is very rigid and cannot rotate (Clayden et al., 2012, p. 105). Thus, the double bond in the hydrocarbon chain of Na-oleate cause rigidity, which then affects the conformation of the surfactant molecule. On the other hand, the hydrocarbon chain of Na-stearate has no double bonds, which means that the hydrocarbon chain is very flexible. It is reasonable that a surfactant molecule, which has more flexible hydrocarbon chain, has higher CMC value compared to a surfactant molecule, which has rigid hydrocarbon chain, since it is presumable that flexible hydrocarbon chain shelf the hydrophilic head slightly better compared to the rigid one. Alawi and Akhter (2011) studied the CMC of sodium surfactants in aqueous solution. They determined the CMC of Na-oleate and Na-stearate in water at 22°C; the CMCs were $10.5 \times 10^{-3} \pm 0.060 \times 10^{-3}$ M and $8.0 \times 10^{-3} \pm 0.037 \times 10^{-3}$ M, respectively. The CMC of Na-oleate was slightly higher compared to the Na-stearate and they concluded that the introduction of a double bond in a surfactant leads to increased CMC. However, the micelles in rapeseed oil are inverse micelles, so it is reasonable to assume that this phenomenon happens vice versa.

In addition, the CMC of sodium soaps are significantly lower compared to the ones determined in water. This is reasonable, since the solubility of the sodium soaps is much lower in rapeseed oil compared to water. Topallar et al. (1997) determined the CMC of Na-stearate in mixed solvent of benzene and methanol (60/40 vol.-%) at 25°C. They observed that the CMC of Na-stearate was 6.1×10^{-3} M, which is quite close to the CMC in water determined by Alawi and Akhter (2011). Moreover, Akhter and Alawi (2003) determined the CMC of ionic surfactants in various organic solvents (formamide (FA), N-methylformamide (NMF) and N,N-dimethylformamide (DMF)) with electrical conductivity and surface tension measurements at 25°C. They observed that the CMCs were significantly lower in FA and in NMF and slightly lower in DMF compared to water. For Na-stearate, the CMCs were 6.2×10^{-4} M, 4.4×10^{-4} M and 1.14×10^{-3} M, respectively. The CMCs in rapeseed oil are even lower, since the solubility of the sodium soaps is very low in rapeseed oil. Thus, it is noteworthy that the CMC values

significantly vary with the media used, since the solubility of the surfactant varies with the polarity of the media used.

No colour changes from yellowish to greenish were observed for Ca-stearate and Fe-stearate and thus, no CMC could be detected. Hence, Ca-stearate and Fe-stearate might not produce RMs in rapeseed oil according to the TCNQ experiments. This could be due to the valence of ionic head groups and the increasing amount of hydrocarbon chains. In aqueous media, di-valent or trivalent counter-ion valence produces a sharp decrease in the CMC when compared to monovalent valence (Terence, 2010). For example, Topallar et al. (1997) determined the CMC of mono-, di-, tri- and tetravalent stearates in the mixture of benzene and methanol (60/40 vol.-%) at 25°C. The CMC decreased with increasing counter-ion valence and the values were 6.1 x 10^{-3} M, 5.3 x 10^{-3} M, 4.9 x 10^{-3} M and 4.2 x 10^{-3} M for sodium, calcium, aluminum and tin stearates, respectively. However, this is not the case in rapeseed oil, since di- and trivalent surfactants have lower HLB value compared to the monovalent surfactants, which means that the solubility of soaps increases with increasing counter-ion valence in nonpolar media. This was also observed when stock solutions were prepared; the solubility of the soaps increased with increasing counter-ion valence. In addition, di- and tri valence soaps have two and three fatty acids attached, respectively, which form a shield around a hydrophilic head and thus the hydrophilic head is sheltered. Thus, it is reasonable to assume that increasing counter-ion valence could increase the CMC of surfactant or even dissolve a certain amount of the soap molecules in nonpolar media. However, no CMC could be detected in the studied concentrations and higher concentrations of Ca- and Fe-stearate could not be dissolved in the oil. Therefore, it is possible that di- and trivalent soaps stay in its initial form in studied concentrations.

3.2.3 The effect of free-fatty acid on critical micelle concentration of soaps

The addition of oleic acid to the medium significantly affected the formation of reverse micellar structures for sodium soaps. Two different amounts of oleic acid were investigated: 50 wt.-% and 5 wt.-%. When 50 wt.-% of oleic acid was added to the media, no color changes were observed. Hence, no micelle formation was observed in rapeseed oil: oleic acid (50/50 wt.-%) media according to the TCNQ experiments. This is reasonable, since already the addition of 20 wt.-% of oleic acid has been observed to interfere the formation of lecithin micelles (Lehtinen et al., 2017) and thus, it is logical that sodium soaps did not form micelles when 50 wt.-% of oleic acid was added to the media. No color change was observed for sodium soaps

with 5 wt.-% of oleic acid either (Figure 18) and hence no micelles could be detected in these experiments with studied concentrations. In addition, Ca- and Fe-stearate did not form micelles in rapeseed oil: oleic acid (50/50 wt.-%) media, which is logical since they were not forming micelles in rapeseed oil either. Thus, they were not studied with 5 wt.-% of oleic acid. The exact soap concentrations with the presence of oleic acid are presented in Appendix 2.

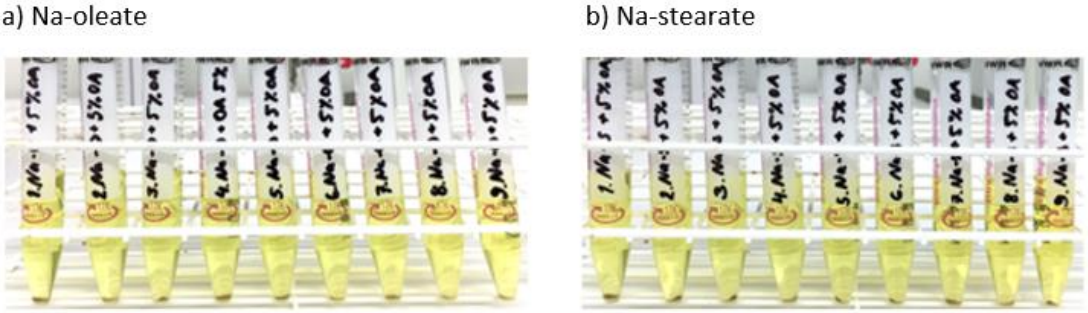


Figure 18. Photographs of the samples after TCNQ addition and centrifugation at various soap concentrations (left to right: lower to higher concentration) in rapeseed oil with 5 wt.-% of oleic acid.

Oleic acid significantly increased the solubility of the soaps, which probably hampered the formation of sodium soap micelles. Lehtinen et al. (2017) studied the effect of oleic acid on the formation of lecithin RMs in rapeseed oil. The similar behavior of increasing solubility with increasing oleic acid concentration was observed; they found that an increase of oleic acid concentration from 0 wt.-% to 20 wt.-%, increased the CMC of lecithin from 6.7 x 10^{-4} M to 2.4 x 10^{-3} M at 70°C. However, no significant changes in the CMC of lecithin was observed with low concentration of oleic acid (< 5 wt.-%) and above 20 wt.-% no CMC was detected. In addition, Kittipongpittaya et al. (2014) observed also that FFAs increased the CMC of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in stripped corn oil. CMC value of DOPC increased from 400 to 1,000 µmol/kg with increasing concentrations of oleic acid (0.5 to 5 wt.-%). No significant differences between FFAs (myristoleic, oleic, elaidic, linoleic and eicosenoic) were observed, even though they had different chain length, configuration and number of double bonds.

Nikifordis et al. (2015) studied the assembly behavior and gel formation of different compositions of an unsaturated FA, oleic acid, and Na-oleate in sunflower oil as discussed in Chapter 2.4.2. They observed the same phenomenon of increased solubility and concluded that the

increased solubility inhibits the extensive aggregation of Na-oleate. Hence, it can be concluded that even small amount of FFA increases the solubility of the soaps in rapeseed oil, which either significantly increase the CMC or prevent the formation of RMs of sodium soaps.

3.2.4 The effect of lecithin on critical micelle concentration of soaps

The effect of lecithin on the CMC of sodium soaps was studied in rapeseed oil. The addition of lecithin was observed to increase the CMC of sodium soaps in rapeseed oil. The CMC values for sodium soaps, sodium soaps with 3.5 x 10^{-5} (mol/l) lecithin and the CMC value of lecithin in rapeseed oil determined by Tiittanen (2016) are presented in Table 10. The graphs showing the effect of lecithin on the CMC of sodium soaps are presented in Figure 19. The graphs showing absorbance as a function of soap concentration are presented in Appendix 3. The water content in the samples were ca 7.6 x 10^{-3} (mol/l). The experiments with lecithin addition to sodium soaps were repeated and the results were reproducible.

Table 10. The CMC values for Na-oleate, Na-stearate, sodium soaps plus lecithin and lecithin in rapeseed oil.

Component	**CMC of soap (mol/l)**
Na-oleate	$4.4 \times 10^{-5} \pm 1.2 \times 10^{-5}$
Na-stearate	$7.1 \times 10^{-5} \pm 2.0 \times 10^{-5}$
Na-oleate + 3.5 x 10^{-5} (mol/l) lecithin	$1.1 \times 10^{-4} \pm 2.3 \times 10^{-5}$
Na-stearate + 3.5 x 10^{-5} (mol/l) lecithin	$1.3 \times 10^{-4} \pm 1.9 \times 10^{-5}$
Lecithin	$9.9 \times 10^{-4} \pm 2.4 \times 10^{-5}$

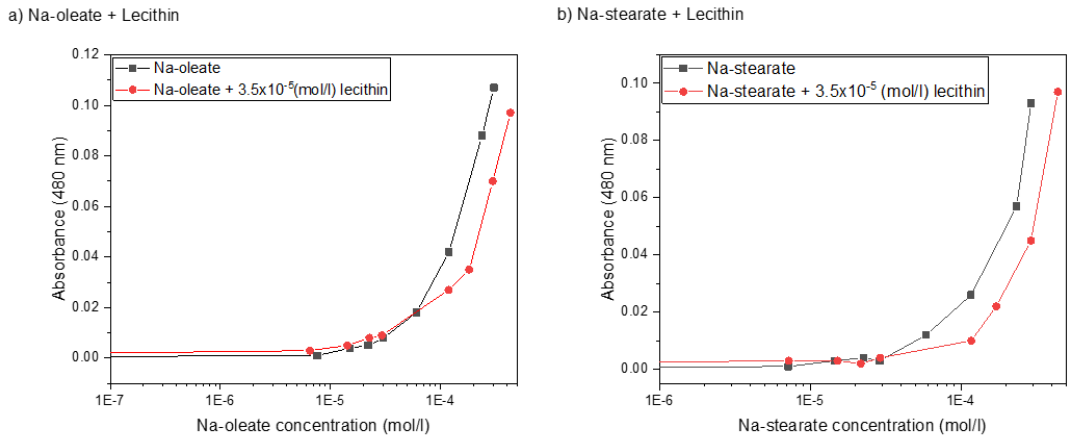


Figure 19. The effect of lecithin on the CMC of sodium soaps in rapeseed oil.

The addition of 3.5 x 10^{-5} (mol/l) lecithin increased significantly the CMC values of sodium soaps. This is probably because the addition of lecithin increases the solubility of sodium soaps in oil. This is reasonable, since soy lecithin has lower HLB value (8) (Barnes and Gentle, 2011, p.167) compared to the sodium soaps (18), which means that lecithin is more soluble in rapeseed oil. Tiittanen (2016) observed the same phenomena of increasing CMC, when introducing a more soluble surfactant (monoglyseride) to lecithin RMs in rapeseed oil. In addition, the increases in the CMC values are logical, since the CMC of lecithin (9.9 x 10^{-4} ± 2.4 x 10^{-5}) is higher compared to the CMC of sodium soaps. There is a slight difference in the CMC values, which is probably due to the double bond in hydrocarbon chain of Na-oleate as discussed in Chapter 3.2.2.

3.2.5 The effect of salts on critical micelle concentration of soaps

The effect of NaCl on the CMC of sodium soaps was studied in rapeseed oil. Varying concentrations of NaCl (0 - 7.6 x 10^{-5} M) was added via a constant amount of liquid NaCl (0.005 g), since salt did not dissolve itself to the oil. Five different salt amounts were studied for Na-oleate and three different for Na-stearate. The addition of NaCl was observed to slightly decrease the CMC of sodium soaps in rapeseed oil. In addition, the addition of pure water (0.005 g) was observed to induce the formation of soap micelles. Figure 20 presents the decrease in CMC values of sodium soaps. The graphs showing absorbance as a function of soap concentration are presented in Appendix 4 and the exact CMC values of sodium soaps + NaCl are presented in Appendix 5. The results were reproducible.

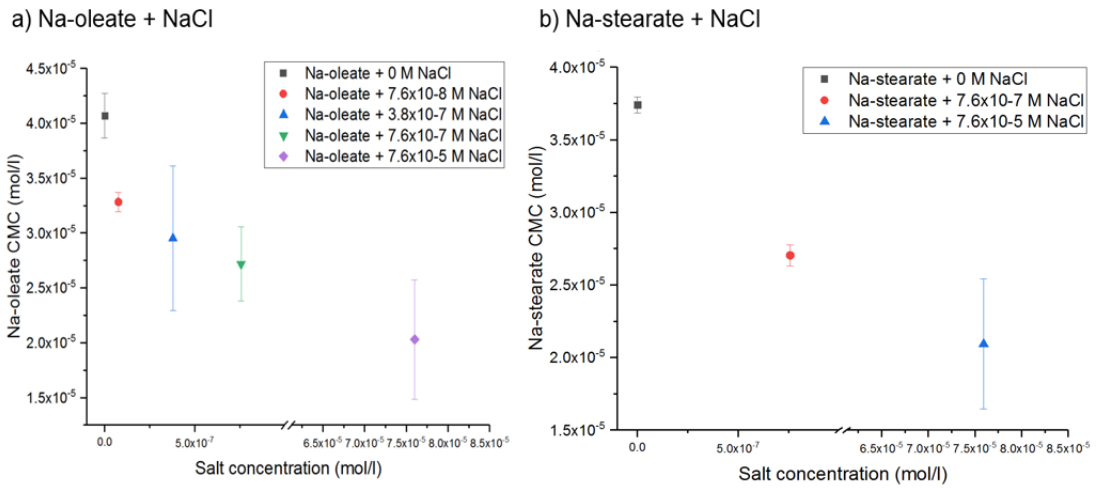


Figure 20. The effect of NaCl on the CMC of Na-oleate (a) and Na-stearate (b).

The addition of NaCl seems to induce the micelle formation of sodium soaps. Both soaps behave quite similarly and no significant differences are observed between soaps. It seems like the greatest decrease happens with small amounts of salt (from 7.6 x 10-8 - 7.6 x 10^{-7} M) and then the decrease seems to slow down. Wan and Poon (1969) observed that anionic salts decreased the CMC of cationic cetrimonium bromide in liquid paraffin. Various cations showed only slight effect on the CMC of cetrimonium bromide. However, there are very few studies on the effect of electrolytes on the CMC of surfactants in non-aqueous media. It is well known that salt addition decreases the CMC of a surfactant in aqueous media as discussed in Chapter 2.3.2. This happens because the additional electrolytes reduce the electrostatic repulsions between the surfactant head groups (Terrence, 2010). As, salt is insoluble in oil, the salt exists only in the water droplets inside the RMs. Thus, the same phenomena of reducing electrostatic repulsions between the surfactant head groups could happen in nonpolar media, which would induce the RM formation. Fathi et al. (2012) observed that salt additions cause a decrease in the average RM size. They concluded that the decrease in the micelle size was direct consequence of the electrical double layer thickness: the layer became thinner as salt concentration increased, which allows the charged head groups to move into closer proximity to each other. Thus, it is presumable that increasing ionic concentration decreases the average micelle size in nonpolar media, which could lead to induced RM formation with lower surfactant concentrations.

3.2.6 Summary of the soap reverse micelle formation in rapeseed oil

Figure 21 represents the summary of the CMC values of soaps and the effect of various additives on the CMC of sodium soaps; Na-oleate and Na-stearate are forming RMs in rapeseed oil according to the TCNQ-experiments. Na-oleate has slightly lower CMC compared to the Na-stearate. Ca- and Fe-stearate are not forming micelles in rapeseed oil according to the TCNQ-experiments. The addition of free-fatty acid (oleic acid (OA)) prevents the formation of sodium soap micelles. The addition of lecithin increases, while the addition of salt decreases the CMC of sodium soaps. It is noteworthy that the CMC values obtained are not very accurate, because it is very person-dependent how the linear fittings are determined in the graphs showing absorbance as a function of surfactant concentration. However, the results are good for approximating the decimal scale in which the CMCs are found and in which direction the additives lead.

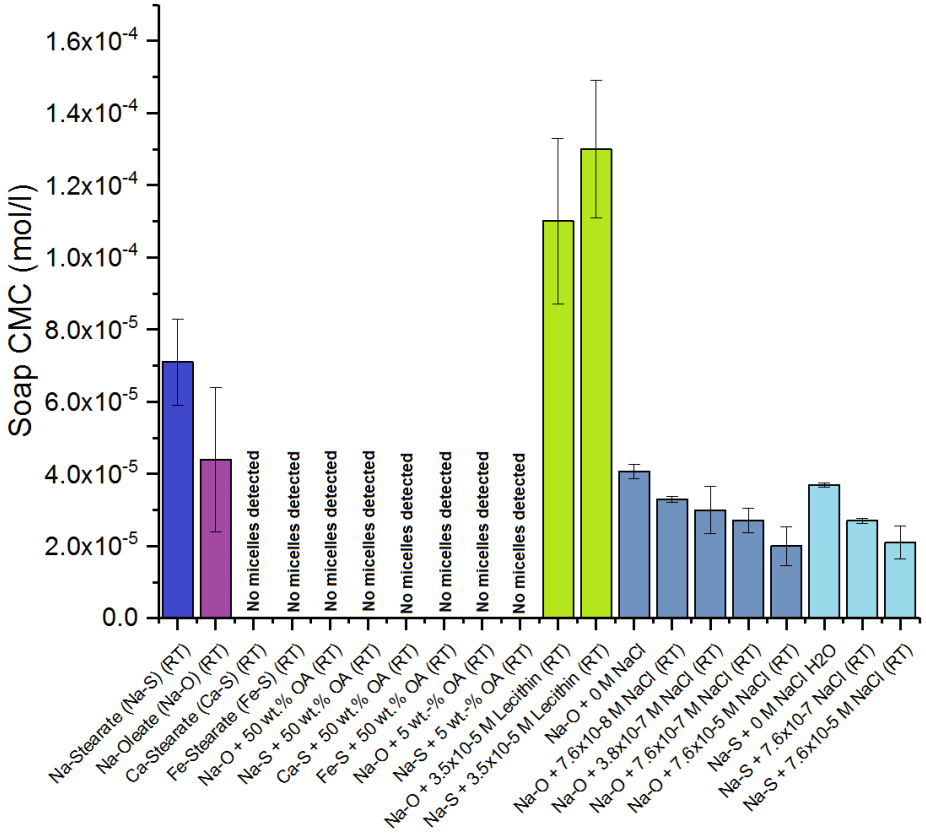


Figure 21. The summary of the CMC values of soaps and the effect of various additives on the CMC of sodium soaps.

3.2.7 The effect of soaps on critical micelle concentration of lecithin

In addition to soap experiments, the effect of all four soaps: Na-oleate, Na-stearate, Ca-stearate and Fe-stearate, on the CMC of lecithin was studied in rapeseed oil with the same molar concentration. Two different soap concentrations were tested: 6.0×10^{-6} M and 2.0×10^{-5} M. The addition of soaps seemed to decrease the CMC of lecithin; however, the results were not that distinct. The color changes were irregular with higher amount (2.0×10^{-5} M) and odd orange color was observed with lower amount (6.0×10^{-6} M) of soap added in lecithin system as presented in Appendix 6. The irregular color changes could indicate unreliable results or different behavior of lecithin-soap mixed micelles than expected. This means that lecithin is probably forming some kind of mixed micelles with soaps, even in very low lecithin concentrations. It is possible that in low lecithin concentrations, few molecules of lecithin participate in soap RM formation, while in higher lecithin concentrations few molecules of soaps participates in lecithin RM formation. It is also possible that too high soap concentrations (2.0×10^{-5} M) were used and thus, soaps could be forming almost independent micelles. However, the absorbance increased logically in every sample series and the CMC could be calculated. The effect of soaps on the CMC of lecithin is presented in Table 11 and Figure 22.

Table 11. The effect of added soap (6.0×10^{-6} M and 2.0×10^{-5} M) on the CMC values of lecithin.

Soap	**CMC of lecithin (mol/l) + 6.0×10^{-6} M soap**	**CMC of lecithin (mol/l) + 2.0×10^{-5} M soap**
Na-oleate	$6.9 \times 10^{-4} \pm 3.1 \times 10^{-5}$	$5.6 \times 10^{-4} \pm 6.4 \times 10^{-5}$
Na-stearate	$8.7 \times 10^{-4} \pm 2.6 \times 10^{-4}$	$2.2 \times 10^{-4} \pm 1.4 \times 10^{-4}$
Ca-stearate	$9.3 \times 10^{-4} \pm 3.9 \times 10^{-5}$	$3.9 \times 10^{-4} \pm 7.7 \times 10^{-5}$
Fe-stearate	-	$3.0 \times 10^{-4} \pm 5.3 \times 10^{-5}$

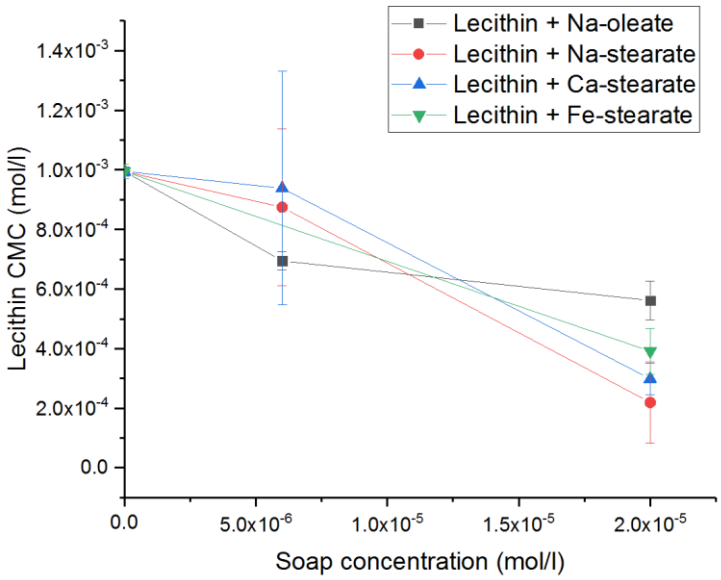


Figure 22. The effect of soaps on the CMC values of lecithin.

The biggest effect on the CMC of lecithin with lower amount of soap addition (6.0 x 10^{-6} M) was observed with Na-oleate, which is reasonable, since Na-oleate had lowest CMC value as determined in Chapter 3.2.2. However, no significant effect on the CMC of lecithin was observed with lower amount of Na-stearate and Ca-stearate (6.0 x 10^{-6} M). Thus, Fe-stearate was not studied with lower concentration. With higher amount of soap addition, Na-oleate had the smallest effect, while Na-stearate had the biggest effect on the CMC of lecithin. It is logical that sodium soaps decrease the CMC of lecithin, since they are more surface-active compared to the lecithin. However, it is unexpected that Na-stearate would have bigger effect on the CMC of lecithin compared to the Na-oleate, since Na-oleate had slightly lower CMC, which would indicate higher surface-activity. Ca-stearate and Fe-stearate was also observed to decrease the CMC of lecithin, even though no micelle formation was detected for these soaps in studied concentrations in rapeseed oil. Hence, it seems like Ca-stearate and Fe-stearate have the ability to form mixed micelles with lecithin.

Since it is presumable that lecithin forms mixed micelles with soaps, it is possible that the micelle structure is changed from cylindrical to some other micellar structure, which thus could affect the CMC. Njauw et al. (2013) studied the molecular interactions between lecithin and bile salts/acids and their effect on reverse micellization in n-decane. They observed that bile salts could transform lecithin RMs into wormlike micelles. It was demonstrated that the positions of hydrogen bonds, which form between bile salts/acids and lecithin are the key to

modulating the self-assembly. In addition, Nikifordis and Scholtenab (2013) studied the mixed self-assembly of lecithin and α- tocopherol in sunflower oil. They observed that the addition of α- tocopherol to the mixture of lecithin and oil induced the formation of cylindrical wormlike micelles. Thus, it is possible that some other micellar structure is formed with addition of soaps, however, this needs to be confirmed with for example SAXS or Cryo-TEM.

3.2.8 Filtration experiments

The filterability of nine different oil samples were tested. The filtration times of the samples and the micelle structures are presented in Table 12. Figure 23 presents the flux of the samples as a function of time (min). The micelle structure of Na-stearate was studied using SAXS and cryo-TEM, and no lamellar structures were observed, which could indicate that sodium-stearate forms normal spherical RMs.

Table 12. Filtration times of the samples. Exact amounts of sample concentrations are presented in Table 6. Lehtinen et al. (2017) determined lecithin micelle structures and soap micelles are expected structures.

Sample number	Sample name	Filtration time (min) per 150 g	Micelle structure
1	Reference (Rapeseed oil)	2.5	No micelles
2	Lecithin below CMC	3.0	No micelles
3	Lecithin above CMC	4.5	Cylindrical lecithin RMs
4	Lecithin above CMC (high water content)	4.5	Lamellar
5	Lecithin above CMC + 5 wt-% oleic acid	4.5	Lamellar
6	Lecithin above CMC + 15 wt-% oleic acid	3.5	No micelles
7	Na-stearate below CMC	3.0	No micelles
8	Na-stearate above CMC	3.0	RMs
9	Na-stearate above CMC (high water content)	3.0	RMs

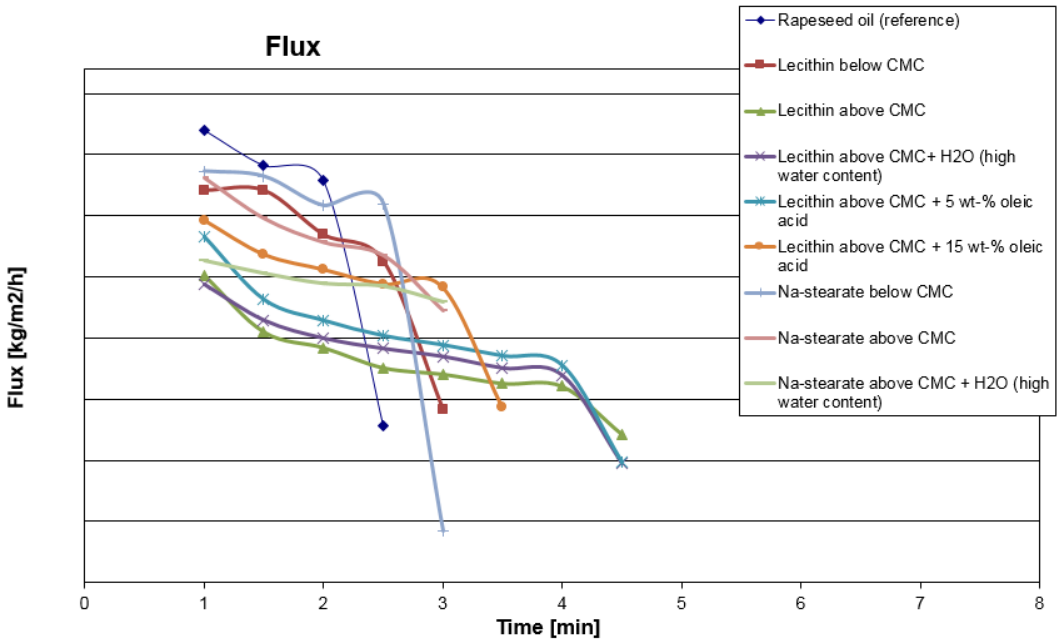


Figure 23. The flux of the samples as a function of time (min).

It is expected that the filtration time increases if the RMs are present. It was observed that the filtration time almost doubled when lamellar or cylindrical lecithin micelles were present. In other words, when lamellar or cylindrical lecithin RMs are present, the filtration time becomes almost twice slower. No differences were observed for lamellar and cylindrical micelles in filtration time. The introduction of FFA (15 wt.-%) speeds up the filtration for one minute compared to the sample, which has lower amount of FFA (5 wt.-%). No differences was observed for Na-stearate below or above the CMC, which could be due to the low soap concentrations. However, the filtration time increased by half a minute when compared to the reference sample. In addition, no differences were observed between sodium soap samples when water was present, so it seems that high amount of water does not affect the filtration time of the sodium soap RMs. It can be concluded that the most significant effect on filtration time of oil occurs if the lamellar or cylindrical lecithin RMs are present, whereas soap RMs does not affect significantly on the filtration time.

Savoirte et al. (2009) observed the same phenomena of retarding effect of phospholipids on filtration: they studied the effect of phospholipids on crude linseed oil filtration at 20°C. They investigated the addition of lecithin between 0 to 10 % and stated that the addition of lecithin (up to 2 %) decreases the filtration rate and increases the cake resistance. They concluded that lecithin molecules would bind particles and the lecithin/particles combination would build a cake, which would gradually block the oil flow. This would support the findings that

lecithin RMs slows the filtration time. On the contrary, Savoirte et al. (2009) also observed that low lecithin concentration (from 0.5 to 2 wt.-%) was observed to improve filtration as it decreases cake resistance. In addition, the increased temperature (50°C) was observed to increase the filterability.

4. Conclusions

The CMC of various soaps, Na-oleate, Na-stearate, Ca-stearate, Fe-stearate, in rapeseed oil was determined using TCNQ solubilization method (Kanamoto et al., 1981). The effect of FFAs (oleic acid), lecithin and NaCl on the CMC of sodium soaps were studied and the effect of soaps on the CMC of lecithin were investigated. In addition, the effect of various micellar structures on filterability of oil was studied. Na-oleate and Na-stearate were observed to form RMs in rapeseed oil according to the TCNQ-experiments; Na-oleate had slightly lower CMC ($4.4 \times 10^{-5} \pm 1.2 \times 10^{-5}$) compared to Na-stearate ($7.1 \times 10^{-5} \pm 2.0 \times 10^{-5}$), which was concluded to be due to the double bond in its hydrocarbon chain. No micelle formation was detected for Ca-stearate and Fe-stearate in studied concentrations. It is possible that Ca- and Fe-stearate do not form micelles at all, since di- and tri valence soaps have two and three FAs attached, respectively, which form a shield around a hydrophilic head and thus the hydrophilic head is sheltered. It is also possible that increasing counter-ion valence increases the CMC of soaps, since the solubility of surfactant increases with increasing counter-ion valence in nonpolar media. To confirm this, more research needs to be done in this area, for example, in another nonpolar media. It was observed that even small addition of FFA (5 wt.-%) increases the solubility of soaps, which probably inhibited the formation of sodium soap RMs or increased significantly their CMC. Even lower amount of FFA additions, for example 0.5, 1, 2 wt.-%, needs to be studied in order to understand in which FFA concentrations soap RM formation is still happening. The addition of lecithin (3.5×10^{-5} M) increased significantly the CMC of Na-oleate and Na-stearate ($1.1 \times 10^{-4} \pm 2.3 \times 10^{-5}$ M and $1.3 \times 10^{-4} \pm 1.9 \times 10^{-5}$ M, respectively), which happens most probably because lecithin increases the solubility of soaps. The increasing NaCl amount was observed to decrease the CMC of sodium soaps. The induced micelle formation happens presumably because increasing salt concentration reduces the electrostatic repulsions between the surfactant head groups, which allows the surfactant molecules to move into closer proximity to each other. However, more research needs to be

done with higher concentrations of salts to confirm this phenomenon. The addition of soaps (Na-oleate, Na-, Ca and Fe-stearate, 2.0 x 10^{-5} M) seemed to decrease the CMC of lecithin; however, the results were not that distinct since irregular color changes were observed. This was probably because too high soap concentrations were used. However, no significant differences were observed in the CMC of lecithin with lower concentration of soap addition (6.0 x 10^{-6} M). Thus, more research needs to be done between studied soap concentrations in order to get reliable results. In addition to CMC investigation, it was observed that the most significant effect on filtration time of oil occurred in the presence of lamellar or cylindrical lecithin RMs; the filtration times became almost twice slower. Soap RMs did not affect significantly on the filtration time of oil. Thus, it can be concluded that lamellar and cylindrical lecithin RMs are critical when refining oil.

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Appendix

Appendix 1. The exact soap concentrations in samples for determine the CMC of soaps in rapeseed oil.

Sample	Na-oleate (mol/l)	Na-stearate (mol/l)	Ca-stearate (mol/l)	Fe-stearate (mol/l)
1	0	0	0	0
2	7.6×10^{-6}	7.2×10^{-6}	6.0×10^{-6}	5.9×10^{-5}
3	1.5×10^{-5}	1.5×10^{-5}	1.4×10^{-5}	1.2×10^{-4}
4	2.2×10^{-5}	2.2×10^{-5}	3.0×10^{-5}	1.8×10^{-4}
5	3.0×10^{-5}	2.9×10^{-5}	4.4×10^{-5}	2.4×10^{-4}
6	6.0×10^{-5}	5.9×10^{-5}	5.8×10^{-5}	3.0×10^{-4}
7	1.2×10^{-4}	1.2×10^{-4}	1.2×10^{-4}	3.6×10^{-4}
8	2.4×10^{-4}	2.3×10^{-4}	1.8×10^{-4}	4.1×10^{-4}
9	3.1×10^{-4}	2.9×10^{-4}	2.4×10^{-4}	4.9×10^{-4}

Appendix 2. The exact soap concentrations with 50 wt.-% of oleic acid (a) and with 5 wt.-% of oleic acid (b).

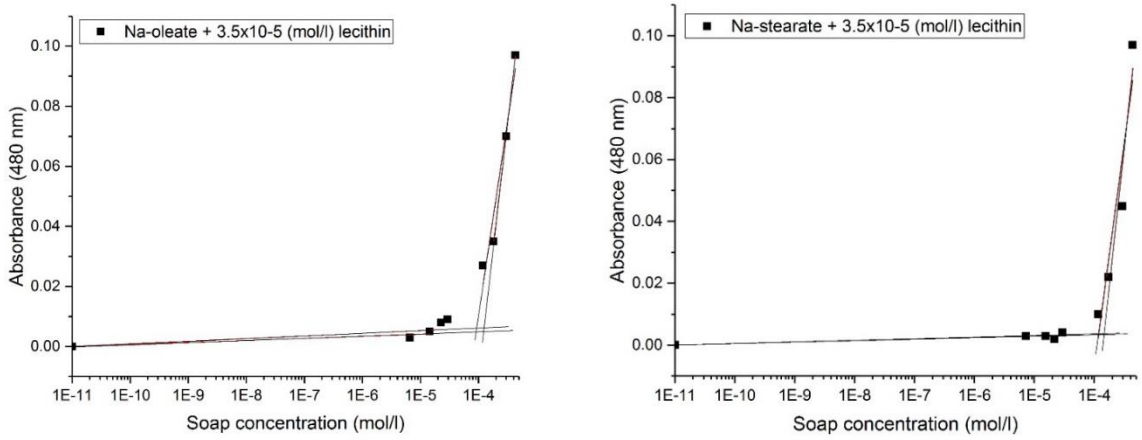
a)

Sample	Na-oleate (mol/l)	Na-stearate (mol/l)	Ca-stearate (mol/l)	Fe-stearate (mol/l)
1	0	0	0	0
2	1.5×10^{-2}	1.3×10^{-2}	8.2×10^{-3}	4.7×10^{-3}
3	7.6×10^{-2}	7.3×10^{-2}	1.5×10^{-2}	9.7×10^{-3}
4	1.5×10^{-1}	1.5×10^{-1}	3.7×10^{-2}	2.5×10^{-2}
5	2.9×10^{-1}	2.5×10^{-1}	7.2×10^{-2}	5.0×10^{-2}
6	0.4	3.8×10^{-1}	1.6×10^{-1}	1.3×10^{-1}

b)

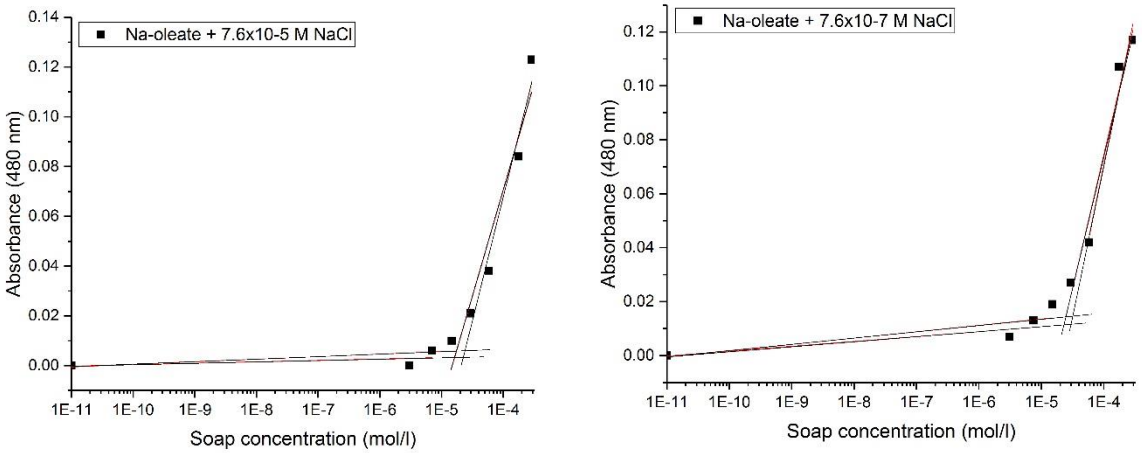
Sample	Na-stearate (mol/l)	Na-oleate (mol/l)
1	0	0
2	6.5×10^{-6}	7.3×10^{-6}
3	1.4×10^{-5}	1.5×10^{-5}
4	2.9×10^{-5}	2.3×10^{-5}
5	5.8×10^{-5}	3.2×10^{-5}
6	1.2×10^{-4}	1.2×10^{-4}
7	2.3×10^{-4}	1.8×10^{-4}
8	2.9×10^{-4}	3.0×10^{-4}
9	4.3×10^{-4}	4.3×10^{-4}

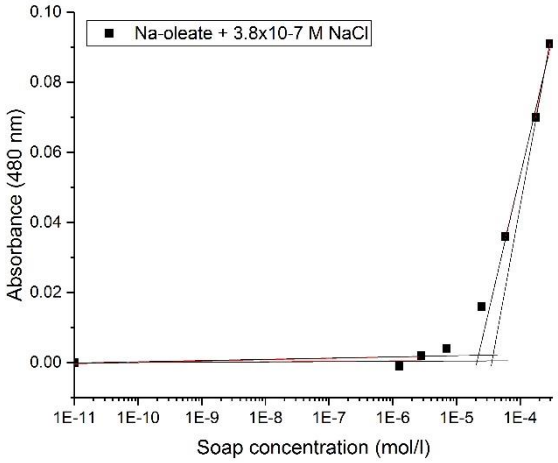
Appendix 3. The CMC determination of sodium soaps + 3.5 x 10^{-5} (mol/l) lecithin in rapeseed oil.

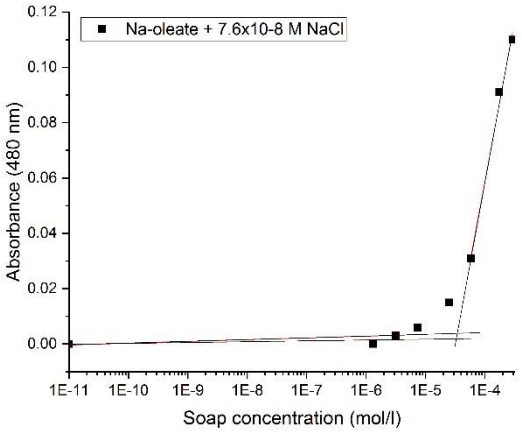


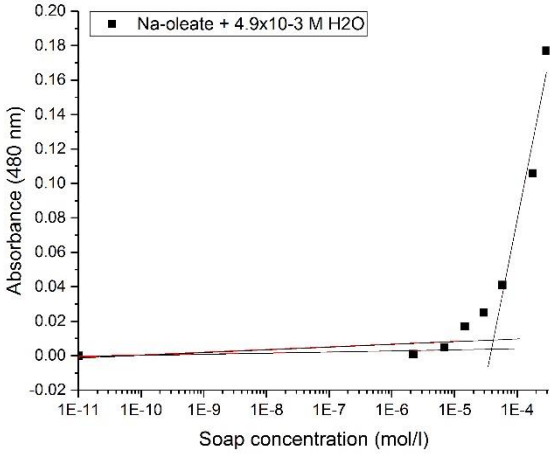
Appendix 4. The CMC determination of Na-oleate + NaCl (a) and Na-stearate + NaCl (b) in rapeseed oil.

a)

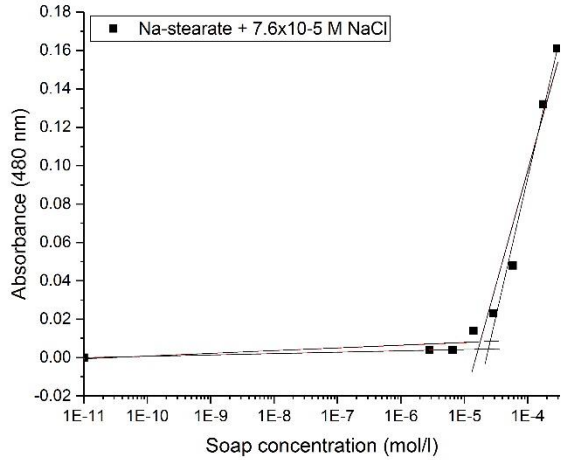


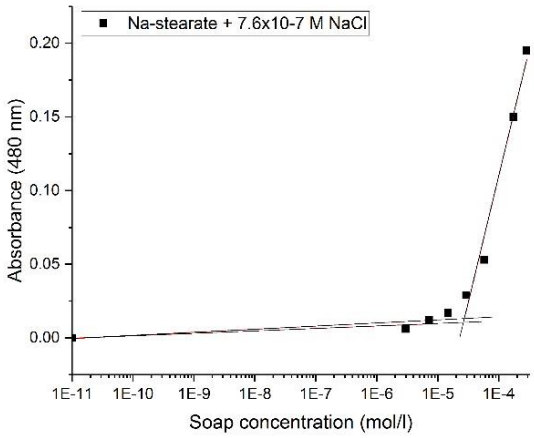

Na-oleate + 3.8x10-7 M NaCl
Absorbance (480 nm)
Soap concentration (mol/l)

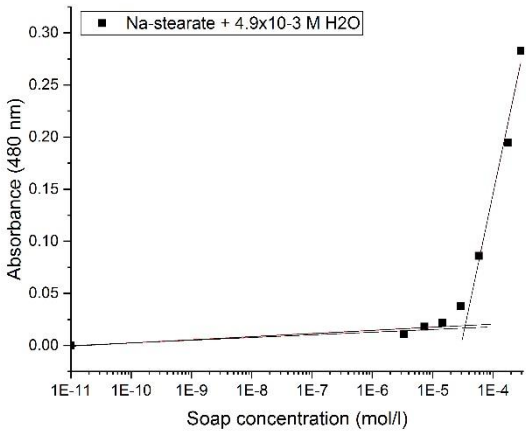

Na-oleate + 7.6x10-8 M NaCl
Absorbance (480 nm)
Soap concentration (mol/l)


Na-oleate + 4.9x10-3 M H2O
Absorbance (480 nm)
Soap concentration (mol/l)

b)


Na-stearate + 7.6x10-5 M NaCl
Absorbance (480 nm)
Soap concentration (mol/l)


Na-stearate + 7.6x10-7 M NaCl
Absorbance (480 nm)
Soap concentration (mol/l)



Appendix 5. The effect of NaCl on the CMC values of Na-oleate (a) and Na-stearate (b).

a)

Component	**CMC (mol/l)**
Na-oleate + 0 M NaCl (4.9 x 10^{-3} M H_2O)	4.1 × 10^{-5} ± 2.0 x 10^{-6}
Na-oleate + 7.6 x 10^{-8} M NaCl	3.3 × 10^{-5} ± 8.6 x 10^{-7}
Na-oleate + 3.8 x 10^{-7} M NaCl	3.0 × 10^{-5} ± 6.6 x 10^{-6}
Na-oleate + 7.6 x 10^{-7} M NaCl	2.7 × 10^{-5} ± 3.4 x 10^{-6}
Na-oleate + 7.6 x 10^{-5} M NaCl	2.0 × 10^{-5} ± 5.4 x 10^{-6}

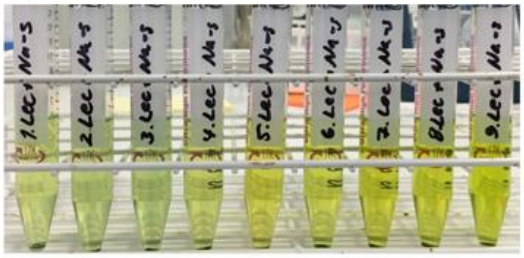
b)

Component	**CMC (mol/l)**
Na-stearate + 0 M NaCl (4.9 x 10^{-3} M H_2O)	3.7 × 10^{-5} ± 5.5 x 10^{-7}
Na-stearate + 7.6 x 10^{-7} M NaCl	2.7 × 10^{-5} ± 7.3 x 10^{-7}
Na-stearate + 7.6 x 10^{-5} M NaCl	2.1 × 10^{-5} ± 4.5 x 10^{-6}

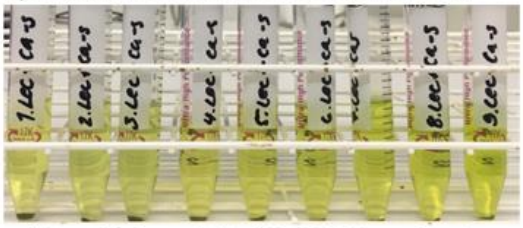
Appendix 6. The effect of TCNQ on varying concentrations of lecithin sample series with higher amount of soap (2.0 x 10^{-5} M) Na-oleate (a), Na-stearate (b), Ca-stearate (c) and Fe-stearate (d) and lower amount of soap (6.0 x 10^{-6} M) Na-oleate (e), Na-stearate (f) and Ca-stearate (g).

a) Lecithin + 2.0x10-5 M Na-oleate

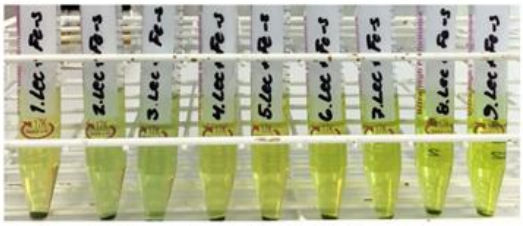
b) Lecithin + 2.0x10-5 M Na-stearate



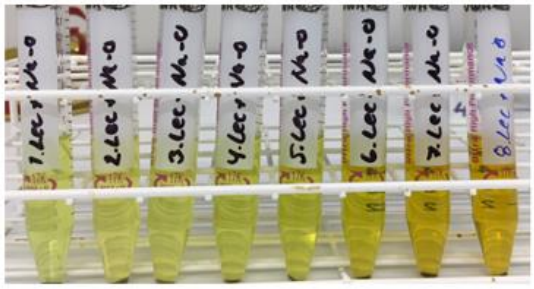
c) Lecithin + 2.0x10-5 M Ca-stearate



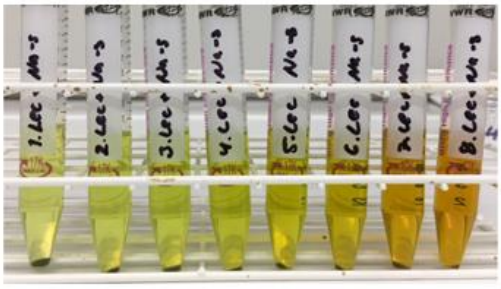
d) Lecithin + 2.0x10-5 M Fe-stearate



e) Lecithin + 6.0x10-6 M Na-oleate



f) Lecithin + 6.0x10-6 M Na-stearate



g) Lecithin + 6.0x10-6 M Ca-stearate